

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
Before the Board of Patent Appeals and Interferences

In re Patent Application of

Atty Dkt. 117-347

C# M#

WILSON et al.

Group Art Unit: 1635

Serial No. 09/787,633

Examiner: Angell

Filed: July 10, 2001

Date: March 26, 2003

Title: TREATMENT OF INFECTION

Assistant Commissioner for Patents  
Washington, DC 20231



AF 11635

#24/E.O.T.  
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Sir:

☐ Correspondence Address Indication Form Attached.

☐ **NOTICE OF APPEAL**

Applicant hereby appeals to the Board of Appeals from the decision dated \_\_\_\_\_ of the Examiner twice/finally rejecting claims \_\_\_\_\_ (\$ 0.00 )

\$ 0.00

☒ An appeal **BRIEF** is attached in triplicate in the pending appeal of the above-identified application (\$ 320.00)

\$ 320.00

☐ Credit for fees paid in prior appeal without decision on merits

-\$ ( )

☐ A reply brief is attached in triplicate under Rule 193(b)

(no fee)

☒ Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s) (\$110.00/1 month; \$410.00/2 months; \$930.00/3 months; \$1450.00/4 months)

\$ 110.00

**SUBTOTAL** \$ 430.00

☒ Applicant claims "Small entity" status, enter 1/2 of subtotal and subtract  
☐ "Small entity" statement attached.

-\$ ( 215.00 )

**SUBTOTAL** \$ 215.00

Less month extension previously paid on

-\$ ( 0.00 )

**TOTAL FEE ENCLOSED** \$ 215.00

Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140**. A duplicate copy of this sheet is attached.

1100 North Glebe Road, 8<sup>th</sup> Floor  
Arlington, Virginia 22201-4714  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100  
BJS:slj

NIXON & VANDERHYE P.C.  
By Atty: B. J. Sadoff, Reg. No. 36,663

Signature: \_\_\_\_\_

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**  
**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

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WILSON et al.

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\* \* \* \* \*

March 26, 2003

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Washington, DC 20231

Sir:

**APPEAL BRIEF**

Pursuant to Rule 192, the appellants respectfully submit the present appeal, in triplicate.

**(1) Real Party in Interest**

The real party in interest is Medical Research Council, 20 Park Crescent, London W1N 4AL, United Kingdom, by way of an Assignment from the appellants which has been recorded in the U.S. Patent and Trademark Office on June 1, 2001, at Reel 011878, Frame 0113.

**(2) Related appeals and interferences**

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#25 / Appeal  
Brief

03/27/2003 CCHAU1 00000001 09787633

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There are no other appeals or interferences known to the appellants, the appellants' legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

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**(3) Status of the claims**

Application PCT/GB99/03180, which designated the U.S. was originally filed with 14 claims (published as WO 00/16758) which were replaced in the international phase with the 11 claims filed November 11, 2000, which were canceled in a Preliminary Amendment filed March 21, 2001, which added new claims 12-19. Claims 14-19 were canceled, and claim 12 was amended in an Amendment filed May 14, 2002. Independent claim 12 and dependent claim 13, which provide a screening method, are pending and are the subject of the present Appeal.

A copy of independent claim 12 and dependent claim 13, which are the subject of the present Appeal, is attached as Appendix A.

**(4) Status of Amendments**

There were no amendments filed in response to the final rejection of July 25, 2002 (Paper No. 17). A Response to Paper No. 17 was filed October 25, 2002. The Response filed October 25, 2002 has been considered by the Examiner and entered and overcome the Section 112, second paragraph, rejection of claims 12 and 13 stated on pages 2-3 of Paper No. 17. See, Advisory Action dated November 18, 2002 (Paper No. 22).

(5) Summary of Invention

The presently claimed invention provides a method of identifying or screening for a compound that inhibits the growth of an organism comprising the *ycf24* gene. See, independent claim 12, lines 1-2, and, for example, page 2, line 28 through page 3, line 13 of the specification and original claims 4-6. The organism of dependent claim 13 is a malaria parasite. See, dependent claim 13, original claim 11, page 4, lines 24-26 and page 1, lines 12-14 of the specification.

The method of independent claim 12 entails at least the process steps of contacting a test compound with the *ycf24* gene product and determining whether the test compound inhibits the activity of or binds to the product, wherein any such binding or inhibition suggests that the compound may inhibit the growth of the organism. See, independent claim 12, original claims 4-6, and page 2, line 28 through page 3, line 13 of the specification.

The presently claimed invention provides a method of screening or identifying compounds which may inhibit growth of an organism harboring the *ycf24* gene, such as a malaria parasite. The appellants have discovered that the *ycf24* gene is essential in organisms harboring the *ycf24* gene and that loss or disruption of the *ycf24* gene is lethal. See, page 2, lines 7-18 of the specification.

The claimed invention was exemplified, in part, with a malaria parasite (*Plasmodium falciparum* (ORF470)) *ycf24* gene sequence (i.e., SEQ ID NO:1). See, page 6, lines 5-21 of the specification.



**(6) Issue**

The following one issue is the subject of the present appeal:

**Whether the specification contains a written description of the invention of claims 12 and 13, as required by 35 U.S.C. §112, first paragraph.**

**(7) Grouping of the claims**

The finally rejected claims do not stand or fall together. Pursuant to Rule 192(c)(7), the appellants will, in the argument section of this Brief, as required by Rule 192(c)(8), explain why the claims of the rejected group are believed to be separately patentable.

**(8) Arguments**

**The specification contains a written description of the claimed invention. Consideration of the following and attached, and reversal of the Examiner's Section 112, first paragraph, "written description" rejection are requested.**

The Examiner has appreciated that the claim recitation "the *ycf24* gene" of line 2 of independent claim 12 is definite. See, page 1 of the Advisory Action dated November 18, 2002 (Paper No. 22), wherein the Examiner indicated the Section 112, second paragraph, rejection of claims 12 and 13 for reciting the same has been withdrawn in view of the appellants Response filed October 25, 2002.

In withdrawing the Section 112, second paragraph rejection of claims 12 and 13 for reciting "the *ycf 24* gene", the Examiner acknowledges that one of ordinary skill in the art will appreciate the metes and bounds of the claimed recitation.

The appellants urge the Board to appreciate that one of ordinary skill in the art will, for similar reasons, recognize that the appellants were in possession of the claimed invention, at least in so far as may be required by Section 112, first paragraph, as construed by the courts and Patent Office guidelines and further described herein.

The appellants have previously submitted the following five literature references with the Response of October 25, 2002<sup>1</sup> in which the authors describe *ycf 24* genes:

Kowallik *et al* (1995) Plant Molecular Biology Reporter, 13, 336-342;

Stirewalt *et al* (1995) Plant Molecular Biology Reporter, 13, 327-332;

Douglas and Penny (1999) J. Mol. Evol. 48, 236-244;

Reardon and Price (1995) Plant Molecular Biology Reporter, 13, 320-326; and

Denny *et al* (1998) Protist, 149, 51-59.

Further copies of these documents are attached as Appendix C.

The Board will appreciate that these documents provide a description of *ycf24* gene sequences (i.e., compounds) from the following separate and distinct organisms:

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<sup>1</sup> The appellants requested in their Response that the Examiner consider this evidence and acknowledge the same by returning the PTO 1449 Form listing the references which was attached to the Response or by returning a PTO 892 Form listing the same. The Examiner has not specifically indicated on the record that these references have been considered by either returning an initialed PTO 1449 Form or a PTO 892 Form listing the references. The Examiner has withdrawn the Section 112, second paragraph, rejection of claims 12 and 13, presumably after consideration of this evidence. The Examiner has also indicated in Paper No. 22 that the "REPLY FILED 22 October 2002" has been considered. These references were included with the Response filed October 25, 2002. The appellants are not aware of a Rely filed October 22, 2002 in the above. A copy of the appellants date-stamped post card receipt from the October 25, 2002 filing is attached as Appendix B. For completeness of the record, and to assure that the present appeal is considered in the most expeditious manner, the Examiner is requested in his Answer to correct and complete the record by specifically indicating that the Response filed October 25, 2002, including the evidence attached thereto, has been considered.

*Odontella sinensis*, *Cyanophora paradoxa*, *Guillardia theta*, *P. purpurea*, and Apicomplexans.

Specifically, Kowallik *et al* describes the chloroplast genome of a chlorophyll-containing alga, *Odontella sinensis* and reports that one of the genes in the genome is "ycf 24 gene" (see Figure 1, page 337, second line from the bottom and page 340). Stirewalt *et al* describes the nucleotide sequence of the cyanelle genome from *Cyanophora paradoxa* and reports that the genome contains ycf 24 (see the fourth row in page 329). Douglas and Penny describes the complete sequence of the plastid genome of the cryptophyte alga, *Guillardia theta*, and reports that it contains ycf 24. Douglas and Penny also confirms that ycf 24 has been identified in other photosynthetic lineages (see Table 1 on page 239). Reardon and Price is a review article about the sequencing of plastid genomes of non-green algae and confirms that ycf 24 has been recognized in such genomes (see the 5<sup>th</sup> item on page 326, i.e., "*P. purpurea*, *C. paradoxa*, *O. sinensis*, others"). Denny *et al* discusses the evidence for a single origin of the 35kB plastid DNA in Apicomplexans and notes that the ycf 24 gene is highly conserved in the plastid across the different species (see page 53, right column, under the heading "The ORF470 Region").

The above-cited and attached references demonstrate that persons skilled in the art consider and considered the term "ycf24 gene" to be clear enough to be used in published scientific articles. They also show that the "ycf 24 gene" has a highly conserved sequence (i.e., compounds) across species and that this allowed persons skilled in the art to recognize the gene (i.e., compounds) in a variety of genomes; the

references demonstrate that, when a genome from a new species was sequenced, any *ycf24* gene was readily identified by its sequence.

The appellants are not claiming in the present application any specific *ycf24* gene sequence but rather the use of a *ycf24* gene sequence or a *ycf24* gene product in a method based on the appellants discovery of the activity of the *ycf24* gene or *ycf24* gene product. The appellants have exemplified the claimed method in the present disclosure by providing *ycf24* sequences of *Plasmodium falciparum* (SEQ ID NO:1), *Synechocystis* PCC6803 (SEQ ID NO:2), and *Escherichia coli* (SEQ ID NO:3). These exemplified *ycf24* gene sequences, taken with the recognized *ycf24* gene sequences of the art, and the whole of the present disclosure, will lead one of ordinary skill in the art to appreciate that the appellants were in possession of the claimed invention at the time the application was filed.

As further evidence of known *ycf24* gene sequences (i.e., compounds), the appellants attached, as an example of the advanced level of skill in the art, as Appendix D, a copy of twenty six (26) *ycf24* gene sequences obtained from the public NCBI database (i.e.,

<http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?CMD=search&DB=nucleotide>

Specifically, *ycf24* gene product sequences from the following organism and/or clones are provided in the attached Appendix D<sup>2</sup>:

Synechocystis sp. PCC 6803 DNA,  
Thermosynechococcus elongatus BP-1,  
Nostoc sp. PCC 7120

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<sup>2</sup> Where the indicated data base entry contained more than the *ycf24* gene and/or gene product, such as the complete genomic sequence, the appellants have provided in the attached, for conservation of paper and space, the sequence obtained by a word search of the term "ycf24" within the retrieved database record. The appellants would be happy to provide a complete copy of the retrieved database record upon the further request of the Examiner and/or the Board.

Synechocystis sp. PCC 6803  
Methanobacterium thermoautotrophicum str. Delta H,  
Toxoplasma gondii apicoplast,  
Odontella sinensis chloroplast,  
Cyanophora paradoxa cyanelle,  
Porphyra purpurea chloroplast,  
Guillardia theta chloroplast,  
Cyanidium caldarium chloroplast,  
Thermosynechococcus elongatus BP-1 section 2/9  
Methanobacterium thermoautotrophicum from bases 1050856 to 1062059  
(section 90 of 148),  
Nostoc sp. PCC 7120 DNA, section 9/19,  
Odontella sinensis complete chloroplast genome,  
Skeletonema costatum chromoplast ycf24 gene, partial,  
Cyanidium caldarium strain RK1 chloroplast,  
Toxoplasma gondii chloroplast,  
Guillardia theta complete plastid genome,  
Porphyra purpurea chloroplast,  
E.coli genomic DNA, Kohara clone #321(38.1-38.4 min.),  
E.coli genomic DNA, Kohara clone #320(37.9-38.3 min.) and  
Cyanophora paradoxa cyanelle.

The following *ycf24* gene products are specifically provided from this database  
(the date of the deposit is indicated in parentheses after the genomic deposit accession  
number):

Synechocystis sp. PCC 6803 DNA gi:1001701 (1995) |:  
MSSTTVKLNQPYKYGFVTNIEADAIPRGLSEDVVRLLISAKKNEPEFMLDFRLRAYRHWLTMAEPTWPA  
VHYPPIDYQDIIYYSA PKQSKKKLES LDEVDPALLETFEKLGIP LSEQKRLSNVAVDAIFDSV SIGTTTFKEKLAEDG  
VIFCSISEALQEHPDLVQKYLGSVVP TADNFFAALNSAVFSDGSFV FIPKGVKCPMELSTYFRINNGDTGQFERTLI  
IAE EGASVSYLEGCTAPMYDTNQLHAAVVELVALDNADIKYSTVQNWYAGDENGKGGIYNFVTKRGLCKGVNSKISW  
TQVETGSAITWKYPSCVLVGDNSVGEFY SIALTNKQQA DTGTKMIHIGKNTR SIIISKGISAGNSANSYRGLVKMG  
PKAQGARNYSQCDSMLIGDRAAANTFPYIQVDNNTAKVEHEASTSKIGEDQLFYFAQRGISEEDAVSMLVSGFCKDV  
LNE LPMEEFAAEADKLLSLKLEGTVG

Thermosynechococcus elongatus BP-1 gi:22297544 (2002)  
MSATVQSLVNQPYKYGFVTPIETETIPKGLNEDIIRLISAKKNEPEFMLEFRLRAYRQWLKMSEPQWPRV  
SYPPINYQDIVYSA PKQKEKLKSLDEVDPVLLETFEKLGIP LSEQKRLTNVAVDAIFDSVSVATTTFREELAKQGII  
FCSISEALQDYPELVQKYLGSVVP IGDNFYAALNSAVFSDGSFVYVPKNTRCPMELSTYFRINNGESGQFERTLI  
DAGSYVSYLEGCTAPMFDTNQLHAAVVELVALDNAEIKYSTVQNWYAGDENGKGGIYNFVTKRGLCLGRNSKISWTQ  
VETGSAITWKYPSCVLVGDNSVGEFY SVALTNHYQQA DTGTKMIHIGKNTRSRIVSKGISAGHSQNSYRGLVKIGPK  
ATGARNYSQCDSMLIGDTAAANTFPYIQVNPTAQVEHEASTSKIGEDQLFYFAQRGISAEDAVSMMISGFCRDVFN  
QLPMEFAVEADRLLSLKLEGSVG

Nostoc sp. PCC 7120 gi:17227497 (2001) \\  
MSATVKTLVNQPYKYGFVTDIEADTIPRGLDEDVRLISTKKNEPEFMLEFRLRAFRQWQKMTPTWPSV  
KYPPIDYQNIYYSA PKQKKAKLNSLDEVDP TLIETFEKLGIP LSEQKRLANVAVDAIFDSVSVATTTFKEKLAKDGV  
IFCSISEALQEHP ELIKKYLGSVVP IADNYFAALNAAVFSDGSFVYIPKGVKCPMELSTYFRINSGDTGQFERTLIV

AEEGSYVSYLEGCTAPMYDSNQLHAAVVELVALDNAEIKYSTVQNWYAGDANGKGGIYNFVTKRGLCQGVNSKISWT  
QVETGSAITWKYPSCVLVDNSVGEFYVALTNNMQQADTGTKMIHIGKNTRSTIIISKGISAGQSSNSYRGLVKINP  
TAKGARNYSQCDSMLIGDNAHANTFPYIQVQNNNTGKVEHEASTSKIGEDQLFFFAQRGISSEDAISMISGFCKDVF  
NQLPMEFAVEADKLLSLKLEGSVG

*Synechocystis* sp. PCC 6803 gi:16329170 (1995)

MSSTTVKNLVNQPYKYGFVTNIEADAIPRGLSEDVRLISAKKNEPEFMDFRLRAYRHWLTMAEPTWPA  
VHYPPIDYQDIIYYSAKQSKKKLESLEVDPALLETFEKLGIPLSEKRLSNVAVDAIFDSVSIIGTTFFKEKLAEDG  
VIFCSISEALQEHDPDLVQKYLGSVVPADNFFAALNSAVFSDGSFVFIPKGVKCPMELSTYFRINNGDTGQFERTLI  
IAEAGASVSYLEGCTAPMYDTNQLHAAVVELVALDNADIKYSTVQNWYAGDENGKGGIYNFVTKRGLCKGVNSKISW  
TQVETGSAITWKYPSCVLVDNSVGEFYIALTNNKQQADTGTKMIHIGKNTRSIISKGISAGNSANSYRGLVKMG  
PKAQGARNYSQCDSMLIGDRAAANTFPYIQVDNNTAKVEHEASTSKIGEDQLFYFAQRGISEEDAVSMLVSGFCKDV  
LNELPMEFAAEADKLLSLKLEGTVG

*Methanobacterium thermoautotrophicum* str. Delta H gi:15678031 (1997)

MLRDTLKKAEKAREKKALYGEDIDLEKFIKEEAGEHEEVTRAKEVPKEVQETLLRVGVDPEERERAGTFI  
QVDQSGICTTCASESIEIMGMNVALDKYSWLKDYMWKAVAVDTDKYTATTALREAEGEMGGYFIRSKPGAREVFPLQ  
ACMFIGDERVMQTAHNIVIAEENSELHIITGCATGEDVSSALHVGVSSEFYLLKKGARITFTMVHNWAEQVEVRPTGI  
MVGDDATYINNYILTSPVKSIQSYPTAYCTGENSRVVFQSILGGQKDSVLDMGSRVILEGRGSSAEMVSRVSKDSS  
QIYSRGLAGRVPEVKHLECHGLVLSDDSMIYAVPELEGSATELEMSHEAAVGKIAEEVVMYLTSRGLTEEEAASM  
IVRGFLSMDITGLPPELAAETKRMLDMSLKGM

*Toxoplasma gondii* apicoplast gi:11496534 (1995)

MKLYKYLNNYNNNTDLFNTVRLIGGLNINMVNKLIFKQDNFIFLYIFRLNALSILNKFQPDWCFYELP  
EFAFDDISYYSIPLNVYTNKNKYKSILSKLGLLEKFSENILLDVIFDSVLLNLTTFFLIKMGFLFLLSFFQSIIIFYP  
YLIFSGLSIVSNTDNFFLTINSIIFNEGSFCFVMKDLNSNINLTTFYRTHSENFAQFERTLIVLSENSKLIYFEGC  
SAPMFLESQHLIAIVELFIKTKANLKYSTIQNWYRGNQLGEGGLYNFTTKRGFCMDKSFNLWIIQIEIGSVITWKYPS  
TYLIGNKSFNSFFSLAMLSDYQVSDTGTKMLHIGKNTKSFILSKLSFNFSFYTYRGLVTIFKTALNSYNYTECNLSL  
LIGCNAFTATIPYTIINNFSAYINQEATISKLELDFLFFLLHRLNLKSTLMILIYGYCYNICSKISFELELEVPLL  
IVARAQKLFY

*Odontella sinensis* chloroplast gi:11467432 (1995)

MTNKSNIKILNTNITKLVNQPYKYGFSTVIEKDIIIEKGLNEDVICLISKKKNEPKFLLEFRLKAFKKWKEM  
KCPDWAQIKFSEIDYQDIIYYSAKVKKKLNSLDEVDPELLKTFEKLGISLTEQKRLANVAIDAVFDSVSIATTFKE  
ELAECGVIFSSISEAIEQYELIEKYLGSVVPIGDNYFSALNSAVFTDGSFCYIPKDTICPLELSTYFRINDQKSGQ  
FERTLIVAEKNSQVSYLEGCTAPQYDSNQLHAAVVELVALENADIKYSTVQNWYAGNNYEGGGIYNFVTKRGLCAGS  
NSKISWTQVETGSNITWKYPSCLLVGDKAKGEFYVALTNNYQQADTGSKMIHVGKNTRSRIVSKGISAGNSKNTYR  
GLVNISNKAIGARNYSQCDSLLIGNLSNANTFPFISVQNPATAKIEHEASTSKIGEEQIFYFLQRGIPKIEKGVLMIS  
GFCQEVFTELPLEFAAEADRLTLKLEGSVG

*Cyanophora paradoxa* cyanelle gi:11467282 (1995)

MVNTQSPKNSGLENLVNQPYKYGLPLIFEIETISKGLTEETIRLISEKKNEPQFMLEFRLQAYRKWLEMS  
NEPEWAHLNYPKINYQDMVYYSAKQKKKLQSLDEVDPTLLETFEKLGIPLTEQKRLANVAIDAVFDSVSVATTFKE  
ELAKEGVIFCPISEAVQKYPDLIKKYLGSVVSTSDNYFSCLNAAVSDGSFCYIPKNVRCPLELSTYFRINNGESGQ  
FERTLIVADEGSYVSYLEGCTAPQFDTNQLHAAVVELVALDNAEIKYSTVQNWYAGDENGKGGIYNFVTKRGLCAGK  
NSKISWTQVETGSAITWKYPSCVLLGDNSIGEFYSVALTNNYQQADTGTKMIHIGKNTRSRISKGISAGHSQNSYR  
GLVKIGPKAVGARNYSQCDSLLIGDNSQANTFPHLQIKNPTAKVEHEASTSKIGEEQIFYFLQRGINAEAAISLIIS  
GFCREVFNLPMEFALEADKLLGLKLEGSVG

*Porphyr*a *purpurea* chloroplast gi:11465652 (1995)

MVNTQNQISQTSDDLIVNQPYKYGFSTTSVESEQFPRGISREVVKLISKKKNEPEYLLNFRLKAYEKWTK  
MKNPKWAHLKHPNIDFNSIIYYAVPKLKKELNSLDEVDPEILDFTFNKLGISLNEQKRLSNVAVDAVFDVSVSIATTFK  
KELAEAGVIFCSISEAIRNYPDLIQKYLGTVPSPGDNYFAALNSAVFSDGSFCYIPPDVTCPLELSTYFRINNEESG  
QFERTLIVADRGSKVSYLEGCTAPQYDTNQLHAAIVELIALDDAEIKYSTVQNWYAGNKDGKGGIYNFVTKRGLCSG  
KNSKISWTQVETGSAITWKYPGILAGDNSQGEFYVALTNNYQEADTGTKMIHIGNNTKSKIISKGISAGKSKNSY

RLVKIGPQSFNSRNYSCDSLLIGQSSQANTFPYIQVQNPTAKVEHEASTSKISEDQIFYFLQRGINLEESVSLMI  
SGFCKDVFNELPMEFAVEADRLLSLKLEGTVG

*Guillardia theta* chloroplast gi:11467607 (1989)

MSDDLKRSRLRELVSQPYKYGFHTDIENEEFPKGLDEDIIEISKLKCEPSYMLDFRLKSYILWKKMSLP  
EWACLTLYLNINYQDIVYYSAPKNSTKLDLEDVDKKILETFDKLGIPLNEQKKLANVAVD AIFDSVSVGTTFKQELS  
NVGVLFCLPSEATNKYSTLVEKYLGSVVPIDGNYFAALNSAVFSEGSFCYIPPNVKCPLELSTYFRINNENSGQFER  
TLIIADFNSYVSYLEGCTAPMYDKNQLHAAVVELIALENAEIRYSTVQNWYSGDTNGKGGIYNFVTKRGLCAGKSSK  
ISWTQVETGSAITWKYPSCILVGEDSVGEFYSVALTNNYQQADTGTKMIHVGRGSKSRIISKGISAGYSKNTRYGQV  
KININALGSINNSQCDSMLIGPYSQANTYPYIQVSNAMSRVEHEASTSKIEEEQLFYFLQRGISVEQAISLLISGFC  
RDVFKLPMEFAVEADKLLSVKLEGTVG

*Cyanidium caldarium* chloroplast gi:11465393 (1996)

MIDRKKSSNIQNILNKPYKYGFSTEQSEEFPGKGINEEIIRLMSHKKQEPDFILKFRLLKAYQIWKMQAP  
DWGHLHHNEINFNDVLCYASPKLEQKKNKAQTISEEILATFEKLGVPKPNKQPKIAVD AFDVDSISFGTTLQKELK  
EQGIIFCSISEAIKAYPNLIKLYLGSIVPAGDNYFAALNSAVFTDGSFCYIPKNIRCPVDLSTYFRINNKEAGQFER  
TLIIADENSFVNYLEGCTAPQFDTNQLHAAVVELICFKNATINYSTVQNWYAGNNKGEQGVYNFVTKRGLCQGENSK  
ISWTQLETGSAITWKYPSCLLKGRSTGEFFSVTLTNNAQEADTGTKMLHFGRQSKSLVISKGISGGVSKNTYRGLV  
KISGSAIYSDNRSQCDSLLIGKSESNTYPNLHVHNSLSKVEHEAFVSRIGEEQIFYFQQRGINIEEALNMIVSGFC  
QDVCNKLPMEFALANKLLNIKLEGSIG

*Thermosynechococcus elongatus* BP-1 section 2/9 gi:22294033 (2002)

MSATVQSLVNQPYKYGFVTPIETETIPKGLNEDIIRLISAKKNEPEFMLEFRLRAYRQWLKMSQWPRV  
SYPPINYQDIVYYSAPKQKEKLKSLDEVDVLLLETFEKLGIPLSEQKRLTNVAVD AIFDSVSVATTFREELAKQGI  
FCSISEALQDYPELVQKYLGSVVPIDGNYFAALNSAVFSDGSFVYVPKNTRCPMELSTYFRINNGESGQFERTLIIA  
DAGSYVSYLEGCTAPMFDTNQLHAAVVELVALDNAEIKYSTVQNWYAGDENGKGGIYNFVTKRGLCLGRNSKISWTQ  
VETGSAITWKYPSCVLVDNSVGEFYSVALTNHYQQADTGTKMIHIGKNTRSRIVSKGISAGHSQNSYRGLVKIGPK  
ATGARNYSQCDSMLIGDTAAANTFPYIQVQNPTAQVEHEASTSKIGEDQLFYFAQRGISAEDAVSMMISGFCRDVFN  
QLPMEFAVEADRLLSLKLEGSVG

*Methanobacterium thermoautotrophicum* gi:2622242 (1997)

MLRDTLLKKAEEKKALYGEDIDLEKFIKEEAGEHEEVTRAKEVPKEVQETLLRVGVDPEERERAGTFI  
QVDQSGICTTCASESIEIMGMNVALDKYSWLKDYMWKAVAVD TDKYTATTALREAEGEMGGYFIRSKPGAREVFPLQ  
ACMFIGDERVMQTAHNIVIAEENSELHIITGCATGEDVSSALHVGVSSEFYLLKKGARITFTMVHNWAEQVEVRPTGI  
MVGDDATYINNYILTSVPKSIQSYPTAYCTGENSRVVFQSILGGQKDSVLDMGSRVILEGRGSSAEMVSRAVSKDSS  
QIYSRGLAGRVPEVKHLECHGLVLSDDSMIYAVPELEGSATELEMSHEAAVGKIAEEVVMYLT SRGLTEEEAASM  
IVRGFLSMDITGLPPELAAETKRMLDMSLKGM

*Nostoc* sp. PCC 7120 gi:17131372 (2001)

MSATVKTLVNQPYKYGFVTDIEADTIPRGLDEDVRLISTKKNEPEFMLEFRLRAFRQWQKMTPTWPSV  
KYPPIDYQNIYYYSAPKQKKAKLNSLDEVDPTLIETFEKLGIPLSEQKRLANVAVD AIFDSVSVATTFFKEKLAKDGV  
IFCSISEALQEHPELIKLYLGSVVPIDNYFAALNAAVFSDGSFVYIPKGVKCPMELSTYFRINSGDTGQFERTLIV  
AEEGSYVSYLEGCTAPMYDSNQLHAAVVELVALDNAEIKYSTVQNWYAGDANGKGGIYNFVTKRGLCQGVNSKISWT  
QVETGSAITWKYPSCVLVDNSVGEFYSVALTNMNOQADTGTKMIHIGKNTRSTIISKGISAGQSSNSYRGLVKINP  
TAKGARNYSQCDSMLIGDNAHANTFPYIQVQNNTGKVEHEASTSKIGEDQLFFFAQRGISED AISMMSGFCRDVFN  
NQLPMEFAVEADKLLSLKLEGSVG

*Odontella sinensis* complete chloroplast gi:1185127 (1995)

MTNKSNIKILNTNITKLVNQPYKYGFSTVIEKDIIEKGLNEDVICLISKKKNEPKFLLEFRLKAFKKWKEM  
KCPDWAQIKFSEIDYQDIIYYSAKPKVKKLNSLDEVDPELLKTFEKLGISLTEQKRLANVAIDAVFDSVSIATTFKE  
ELAECGVIFSSISEAIEQYELIEKYLGSVVPIGDNYFSALNSAVFTDGSFCYIPKDTICPLELSTYFRINDQKSGQ  
FERTLIVAENKNSQVSYLEGCTAPQYDSNQLHAAVVELVALENADIKYSTVQNWYAGNNYEGGGIYNFVTKRGLCAGS  
NSKISWTQVETGSNITWKYPSCLLVGDKAKGEFYSVALTNNYQQADTGSKMIHVGNTRSRIVSKGISAGNSKNTYR  
GLVNISNKAIGARNYSQCDSLLIGNLSNANTFPFISVQNPTAKIEHEASTSKIGEEQIFYFLQRGIPIEKGVLMIS  
GFCQEVFTELPLEFAAEADRLTLKLEGSVG

Skeletonema costatum chloroplast ycf24 gene, partial gi:4210403 (1999)  
AVFTDGSFCYIPKDVICPLDLSTYFRINDQNSGQFERTLIIAEENSKVSYLEGCTAPQYDNNQLHAAIVE  
LIALKNATIKYSTVQNWYSGDQKGQGGVYNFVTKRGLCAGDFSKISWTQVETGSSITWKYPSCVLVGDSAQGEFYSV  
ALTNNYQQADTGTKMIHIGNTRSRIVSKGISA

Cyanidium caldarium strain RK1 chloroplast gi:6466296 (1996)  
MIDRKSSNIQNILNKPYKYGFSTEIQSEEFPGKINEEIIIRLMSHKKQEPDFILKFRLLKAYQIWKMKQAP  
DWGHLHHNEINFNDVLCYASPKLEQKGKNAQTISEEILATFEKLGVPKPNKQPKIAVDAVFDSSISFGTTLQKELK  
EQGIIIFCSISEAIAKAYPNLIKYLGSIVPAGDNYFAALNSAVFTDGSFCYIPKNIRCPVDLSTYFRINNKEAGQFER  
TLIIADENSFVNYLEGCTAPQFDTNQLHAAVVELICFKNATINYSTVQNWYAGNNKGEAGVYNFVTKRGLCQGENSK  
ISWTQLETGSAITWKYPSCLLKGKRSTGEFFSVTLTNNAQEADTGTKMLHFGKQSKSLVSKGISGGVSKNTYRGLV  
KISGSAIYSDNRSQCDSLLIGKGSSENTYPNLHVHNSLSKVEHEAFVSRIGEEQIFYFQQRGINIEEALNMIVSGFC  
QDVCNKLPMFALEANKLLNIKLEGSIG

Toxoplasma gondii chloroplast gi:5231237 (1995)  
MKLYKYLYNNKNTDLFNTVRLIGGLNINMVNKLIFKQDNFIFLYIFRLNALSILNKFQPDWCFYELP  
EFAFDDISYYSIPLNVYTNKNKYKSILSKLGLLEKFSENLILDVIFDSVLLNLTTFFLIKMGFLFSLFFQSIIFYF  
YLIFSYLEGSIVSNTDNFFLTINSIIIFNEGSFCFVMKDLNSNINLTTYFRTHSENFAQFERTLIVLSENSKLIYFEGC  
SAPMFLESQHLIAIVELFIKTKANLKYSTIQNWYRGNQLGEGGLYNFTTKRGFCMDKSFLNWIQIEIGSVITWKYPS  
TYLIGNKSFNSFFSLAMLSDYQVSDTGTKMLHIGKNTKSFILSKLSFNFSFYTYRGLVTIFKTALNSYNYTECNLS  
LIGCNAFTATIPYTIINNFSAYINQEATISKLELDFLFLHRLNLKSTLMILIYGICYNICKISFELELEVPLL  
IVARAQKLFY

Guillardia theta complete plastid genome gi:3602932 (1989)  
MSDDLKRSRLRELVSQPYKYGFHTDIENEEFPKGLDEDIIEKISKLKCEPSYMLDFRLKSYILWKKMSLP  
EWACLTLYLNINYQDIVYYSAPKNSTKLDLEDVDKKILETFDKLGIPLNEQKLANVAIDAVFDSVSVGTTFKQELS  
NVGVLCPLSEATNKYSTLVEKYLGSVVPIGDNYFAALNSAVFSEGSFCYIPPNVKCPLSTYFRINNENSGQFER  
TLIIADFNSYVSYLEGCTAPMYDKNQLHAAVVELIALENAEIRYSTVQNWYSGDTNGKGGIYNFVTKRGLCAGKSSK  
ISWTQVETGSAITWKYPSCILVGEDSVGEFYSVALTNNYQQADTGTKMIHVGRGSKSRIISKGISAGYSKNTYRQV  
KININALGSINNSQCDSMLIGPYSQANTYPYIIVSNAMSRVEHEASTSKIEEEQLFYFLQRGISVEQAISLLISGFC  
RDVFKVCLPMFAVEADKLLSVKLEGTVG

Porphyra purpurea chloroplast gi:1276652 (1995)  
MVNTQNQISQTSDDLIVNQPYKYGFHTTSVESEQFPRGISREVVKLISKKKNEPEYLLNFRLLKAYEKWTK  
MKNPKWAHLKHPNIDFNSIIYYAVPKLKKELNSLDEVDPEILDFTFNKLGISLNEQKRLSNVAIDAVFDSVSIATTFK  
KELAEAGVIFCSISEAIRNYPDLIQKYLGTVPSPGDNYFAALNSAVFSDGSFCYIPPDVCPLELSTYFRINNENSG  
QFERTLIVADRGSKVSYLEGCTAPQYDTNQLHAAIVELIALDDAEIKYSTVQNWYAGNKDGKGGIYNFVTKRGLCSG  
KNSKISWTQVETGSAITWKYPGILAGDNSQGEFYSVALTNNYQEADTGTKMIHIGNNTKSKIISKGISAGKSKNSY  
RGLVKIGPQSFSNRNYSQCDSLLIGQSSQANTFPYIIVQNPTAKVEHEASTSKISEDQIFYFLQRGINLEESVSLMI  
SGFCKDVFNELPMFAVEADRLLSLKLEGTVG



E.coli genomic DNA, Kohara clone #321 gi:1742768 (1996)

MWLWRKLGIGGTMSRNTEATDDVKTWTGGPLNYKEGFFTQLATDELAAGINEEVVRAISAKRNEPEWML  
EFRLNAYRAWLEMEEPHWLKAHYDKLNYQDYSYYSAPSCGNCDDTCASEPGAVQQTGANAFLSKEVEAAFEQLGVPV  
REGKEVAVD AIFDSVSVATTYREKLAEQGIIFCSFGEAIHDHPELVRKYLGTVPVPGNDNFFAALNAAVASDGTFIYV  
PKGVRCPMELSTYFRINAECTGQFERTILVADEDSYVSYIEGCSAPVRDSYQLHAAVVEVIIHKNAEVKYSTVQNW  
PGDNNTGGILNFVTKRALCEGENSKMSWTQSETGSAITWKYPSCILRGDNSIGEFYSVALTSGHQADTGTKMIHIG  
KNTKSTIIISKGISAGHSQNSYRGLVKIMPTATNARNFTQCDSMLIGANCGAHTFPYVECRNNSAQLEHEATTSRIGE  
DQLFYCLQRGISEEDAISMIVNGFCKDVFSELPLEFAVEAQKLLAISLEHVS

E.coli genomic DNA, Kohara clone #320 gi:1742754 (1996)

MWLWRKLGIGGTMSRNTEATDDVKTWTGGPLNYKEGFFTQLATDELAAGINEEVVRAISAKRNEPEWML  
EFRLNAYRAWLEMEEPHWLKAHYDKLNYQDYSYYSAPSCGNCDDTCASEPGAVQQTGANAFLSKEVEAAFEQLGVPV  
REGKEVAVD AIFDSVSVATTYREKLAEQGIIFCSFGEAIHDHPELVRKYLGTVPVPGNDNFFAALNAAVASDGTFIYV  
PKGVRCPMELSTYFRINAECTGQFERTILVADEDSYVSYIEGCSAPVRDSYQLHAAVVEVIIHKNAEVKYSTVQNW  
PGDNNTGGILNFVTKRALCEGENSKMSWTQSETGSAITWKYPSCILRGDNSIGEFYSVALTSGHQADTGTKMIHIG  
KNTKSTIIISKGISAGHSQNSYRGLVKIMPTATNARNFTQCDSMLIGANCGAHTFPYVECRNNSAQLEHEATTSRIGE  
DQLFYCLQRGISEEDAISMIVNGFCKDVFSELPLEFAVEAQKLLAISLEHVS

Cyanophora paradoxa cyanelle gi:1016083 (1995)

MVNTQSPKNSGLENLVNQPYKYGLPLIFEIETISKGLTEETIRLISEKKNEPQFMLEFRLQAYRKWLEMS  
NEPEWAHLNYPKINYQDMVYYSAPKQKKKLQSLDEVDPTLLETFEKLGIPLTEQKRLANVAVD AIFDSVSVATTTFKE  
ELAKEGVIFCPISEAVQKYPDLIKKYLGSVSTSDNYFSCLNAAVFSFGSFCYIPKNVRCPLSTYFRINNGESGQ  
FERTLIVADEGSYVSYLEGCTAPQFDTNQLHAAVVELVALDNAEIKYSTVQNWYAGDENGKGGIYNFVTKRGLCAGK  
NSKISWTQVETGSAITWKYPSCVLLGDNSIGEFYSVALTNRYQQADTGTKMIHIGKNTSRRIISKGISAGHSQNSYR  
GLVKIGPKAVGARNYSQCDSLLIGDNSQANTFPHLQIKNPTAKVEHEASTSKIGEEQIFYFLQRGINAEAAISLIIS  
GFCREVFNLPMEFALEADKLLGLKLEGSV

Clearly, a number of *ycf24* genes and *ycf24* gene products were known and identifiable at the time of the present invention. The specification exemplifies *ycf 24* genes which may have any of SEQ ID NO: 1 (the sequence of the malaria parasite *Plasmodium falciparum*), SEQ ID NO: 2 (the sequence of *Synechocystis* PCC6803) and SEQ ID NO: 3 (the sequence of *E.coli*). See, page 4, lines 32 -34 of the specification, where it is stated that the *ycf 24* gene product is generally "one which can be expressed from the coding region of: (a) the polynucleotide sequence of SEQ ID NO: 1, 2, or 3 ...". The specification further describes that the *ycf 24* gene of the presently claimed invention may be encoded by "polynucleotide which can selectively hybridize to the

coding region of" SEQ ID NO: 1, 2 or 3 (see page 5, lines 2-3 of the specification). An example of hybridization conditions is given at page 5, lines 6-9. Moreover, the specification describes that the *ycf 24* gene may be a malaria gene, a red algal gene, a bacterial gene or an *E.coli* gene. For example, the specification makes clear at page 4, lines 26-31 that the organism may be "*Plasmodium falciparum* [a malaria parasite] ... an alga ... a bacterium ... or *E.coli*".

The present specification therefore provides a functional and a structural description of a number of *ycf24* genes and *ycf24* gene products. The structural similarity of the proteins and DNA sequences of the claims at issue in this appeal are described by reference to the well known identification "*ycf24* gene" and exemplification of a number of sequences falling within the definition.

The Patent Office's "current understanding... regarding the written description requirement of 35 U.S.C. 112, ¶1" (see, 66 FR 1099, Friday, January 5, 2001 (copy attached as Appendix E) states that

"An applicant may show possession of an invention by disclosure of drawings<sup>39</sup> or structural chemical formulas<sup>40</sup> that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole. The description need only describe in detail that which is new or not conventional.<sup>41</sup> This is equally true whether the claimed invention is directed to a product or a process.

An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics<sup>42</sup> which provide evidence that applicant was in possession of the claimed invention,<sup>43</sup> i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.<sup>44</sup> What is conventional or well-known to one of ordinary skill in the art need not be disclosed in detail.<sup>45</sup> If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of

the claims is not explicitly described in the specification, then the adequate description requirement is met.<sup>46n</sup> Id. at 1106.

The indicated footnotes 39-46 further support this "understanding" of the Patent Office based on Federal Circuit, CCPA and other case law as follows:

<sup>39</sup>See, e.g., *Vas-Cath*, 935 F.2d at 1565, 19 USPQ2d at 1118 ("drawings alone may provide a 'written description' of an invention as required by § 112"); *In re Wolfensperger*, 302 F.2d 950, 133 USPQ 537 (CCPA 1962) (the drawings of applicant's specification provided sufficient written descriptive support for the claim limitation at issue); *Autogiro Co. of America v. United States*, 384 F.2d 391, 398, 155 USPQ 697, 703 (Ct. Cl. 1967) ("In those instances where a visual representation can flesh out words, drawings may be used in the same manner and with the same limitations as the specification.").

<sup>40</sup>See e.g., *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus.").

<sup>41</sup>See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805 (source code description not required).

<sup>42</sup>For example, the presence of a restriction enzyme map of a gene may be relevant to a statement that the gene has been isolated. One skilled in the art may be able to determine when the gene disclosed is the same as or different from a gene isolated by another by comparing the restriction enzyme map. In contrast, evidence that the gene could be digested with a nuclease would not normally represent a relevant characteristic since any gene would be digested with a nuclease. Similarly, isolation of an mRNA and its expression to produce the protein of interest is strong evidence of possession of an mRNA for the protein.

For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. For example, unique cleavage by particular enzymes,

isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities, or antibody cross-reactivity may be sufficient to show possession of the claimed invention to one of skill in the art. See *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966 ("written description" requirement may be satisfied by using" such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention").

<sup>43</sup>A definition by function alone "does not suffice" to sufficiently describe a coding sequence "because it is only an indication of what the gene does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. See also *Fiers*, 984 F.2d at 1169-71, 25 USPQ2d at 1605-06 (discussing *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)).

<sup>44</sup>If a claim limitation invokes 35 U.S.C. 112, ¶ 6, it must be interpreted to cover the corresponding structure, materials, or acts in the specification and "equivalents thereof." See 35 U.S.C. 112, ¶ 6. See also *B. Braun Medical, Inc. v. Abbott Lab.*, 124 F.3d 1419, 1424, 43 USPQ2d 1896, 1899 (Fed. Cir. 1997). In considering whether there is 35 U.S.C. 112, ¶ 1, support for a means- (or step) plus-function claim limitation, the examiner must consider not only the original disclosure contained in the summary and detailed description of the invention portions of the specification, but also the original claims, abstract, and drawings. A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, ¶ 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have know what structure, material, or acts perform the function recited in a means- (or step-) plus- function limitation. Note also: A rejection under 35 U.S.C. 112, ¶ 2, "cannot stand where there is adequate description in the specification to satisfy 35 U.S.C. 112, first paragraph, regarding means-plus-function recitations that are not, per se, challenged for being unclear." *In re Noll*, 545 F.2d 141, 149, 191 USPQ 721, 727 (CCPA 1976). See *Supplemental Examination Guidelines for Determining the Applicability of 35 U.S.C. 112, ¶ 6*, 65 FR 38510, June 21, 2000.

<sup>45</sup>See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94.

<sup>46</sup>See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (starting "the description need not be in *ipsis verbis* (i.e., "in the same words"] to be sufficient")." *Id.* at 1109-1110. (Emphasis added.)

As noted above in footnote 40, the Patent Office confirms that the court in *Eli Lilly* found that claims involving generic formula usually indicate with specificity what the generic claims encompass. The court confirmed that one of ordinary skill in the art can usually distinguish such a formula from others and can identify many of the species that the claims encompass. Given these facts, the *Eli Lilly* court concluded that "such a formula is normally an adequate written description."

In the present appeal, the appellants have described, and recited in the claims and specification, a gene and gene product by reference to three nucleic acid and three amino acid sequences, a source of these material, and a well-known identifier (i.e., *ycf24* gene) as evidenced by at least the five references of Appendix C and as exemplified by at least the sequence excerpts from the publicly available NCBI database provided in Appendix D, which allows one of ordinary skill to distinguish the generic formula of the claims from other gene product and gene sequences. One of ordinary skill can identify many species that the claims encompass. Given the conclusions of the *Eli Lilly* court, the appellants submit that the generic formula of the claims on appeal and the specification provide an adequate written description.

The issue before the *Eli Lilly* court, which was not mentioned in the footnote of the Patent Office's "written description" analysis, was whether even more generic statements, "such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA', without more," is an adequate' written description. See, 43 USPQ2d 1406 (emphasis added).

The *Eli Lilly* court found that such a generic recitation was not an adequate written description.

"because it does not distinguish the claimed genus from others except by function. It does not specifically define any of the genes that fall within its definition. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is. See *Fiers*, 984 F.2d at 1169-71, 25 USPQ2d at 1605-06 (discussing *Amgen*)." Id.

As noted above, the claims at issues in the present appeal recite a reference sequence, i.e., the *ycf24* gene, and the specification exemplifies a number of sequences, which allow one of ordinary skill to distinguish the gene and gene product of the claimed invention from other genes and gene products.

The appellants respectfully submit that the present specification demonstrates possession of the claimed invention by, for example, disclosure of reference sequences (i.e., SEQ ID NOs: 1, 2 and 3) and the well-known source of these sequences coupled with the functional characteristics recited in the claims. An ordinarily skilled artisan would have understood the appellants were in possession of the claimed invention at the time of filing, even if every nuance of the claimed invention is not explicitly described in the specification.

Beyond the Patent Office "understanding" of the requirements of the Section 112, first paragraph, written description, requirement, as detailed above, the Patent Office has issued Training Materials

"designed to aid PTO's patent examiners in applying the interim written description... guidelines in a uniform and consistent manner to promote the issuance of high quality patents. The training materials will also assist patent applicants in responding to the PTO when... written description issues are raised during the

examination of a patent application." See, Press Release #00-15, USPTO, March 1, 2000 ([www.uspto.gov/web/offices/com/speeches/00-15.html](http://www.uspto.gov/web/offices/com/speeches/00-15.html)) (copy attached as Appendix F).

#### The Written Description Training Materials

(<http://www.uspto.gov/web/offices/pac/writtendesc.pdf>) offer the following Examples 14

and 18 ("Product by Function" and "Process claim where the novelty is in the method steps"):

#### **"Example 14: Product by Function"**

**Specification:** The specification exemplifies a protein isolated from liver that catalyzes the reaction of **A** → **B**. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following:

substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

#### **Claim:**

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of **A** → **B**.

#### **Analysis:**

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art. A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3.

Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be

noted that “having” is open language, equivalent to “comprising”. The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious. There is actual reduction to practice of the single disclosed species.

The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that applicant was in possession of the necessary common attributes possessed by the members of the genus.

**Conclusion:** The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

**Example 18: Process claim where the novelty is in the method steps.**

**Specification:** The specification teaches a method for producing proteins using mitochondria from the fungus *Neurospora crassa*. In the method, mitochondria are isolated from this fungus and transformed with a mitochondrial expression vector which comprises a nucleic acid encoding a protein of interest. The protein is subsequently expressed, the mitochondria is lysed, and the protein is isolated. The specification exemplifies the expression of beta-galactosidase using the claimed method using a cytochrome oxidase promoter.

**Claim:**

1. A method of producing a protein of interest comprising; obtaining *Neurospora crassa* mitochondria, transforming said mitochondria with a expression vector comprising a nucleic acid that encodes said protein of interest, expressing said protein in said mitochondria, and recovering said protein of interest.

**Analysis:**

A review of the specification reveals that *Neurospora crassa* mitochondrial gene expression is essential to the function/operation



Table I. List of genes and ORFs of the *Odontella sinensis* chloroplast genome. The gene list proceeds clockwise from the LSC boundary of the right-hand inverted repeat (IRa). Genes are listed together with their putative gene products. ORFs with a (G+C)-content less than 30% (ORFs 25, 26a, 26b, 27, 29a, 29b, 41, 44, 46) are designated (A+T)-rich and may be non-coding. Numerical positions include the first nucleotide of the translation initiation codon as well as the last nucleotide of the stop codon. Transcription initiation and termination sites of tRNAs and rRNAs were determined according to multiple alignments and secondary structure models. Genes marked "(-)" are on the complementary strand.

Gene	Gene Product	Coding Sites	
	<i>start IRa</i>		1
<i>trnP</i>	tRNA-Pro(ugg)	267	340
ORF355		478	1545
<i>rrn16</i>	16S ribosomal RNA	2210	3694
<i>trnI</i>	tRNA-Ile(gau)	3761	3835
<i>trnA</i>	tRNA-Ala(ugc)	3838	3910
<i>rrn23</i>	23S ribosomal RNA	3976	6866
<i>rrn5</i>	5S ribosomal RNA	6938	7063
<i>ycf32</i>	ORF36	7318	7428
<i>rpl32</i>	50S ribosomal protein L32	7563	7739
	<i>end IRa</i>	7725	
<i>psaC</i>	PSI, Fe-S polypeptide SU VII, 9 kDa	7825	8073
<i>ycf5</i>	ORF312 (-)	8139	9077
ORF46	(A+T)-rich	9287	9427
<i>rps6</i>	30S ribosomal protein S6	9482	9772
<i>trnN</i>	tRNA-Asn(guu)	9852	9922
<i>thiG</i>	involved in thiamine biosynthesis	9944	10729
<i>clpC</i>	Clp protease, caseinolytic-like (-)	10894	13551
<i>trnC</i>	tRNA-Cys(gca)	13780	13850
<i>trnL</i>	tRNA-Leu(uaa)	13921	14006
<i>rps10</i>	30S ribosomal protein S10 (-)	14207	14518
<i>tufA</i>	elongation factor Tu (-)	14531	15760
<i>rps7</i>	30S ribosomal protein S7 (-)	15831	16307
<i>rps12</i>	30S ribosomal protein S12 (-)	16341	16721
<i>rpl31</i>	50S ribosomal protein L31 (-)	16773	16991
<i>rps9</i>	30S ribosomal protein S9 (-)	17005	17421
<i>rpl13</i>	50S ribosomal protein L13 (-)	17445	17864
<i>rpoA</i>	RNA polymerase $\alpha$ -chain (-)	17893	18831
<i>rps11</i>	30S ribosomal protein S11 (-)	18883	19275
<i>rps13</i>	30S ribosomal protein S13 (-)	19320	19691
<i>rpl36</i>	50S ribosomal protein L36 (-)	19713	19826
<i>secY</i>	preprotein-translocase subunit Y (-)	19866	21143
<i>rps5</i>	30S ribosomal protein S5 (-)	21166	21723
<i>rpl18</i>	50S ribosomal protein L18 (-)	21765	22172
<i>rpl6</i>	50S ribosomal protein L6 (-)	22219	22758
<i>rps8</i>	30S ribosomal protein S8 (-)	22777	23175

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<i>rpl5</i>	50S ribosomal protein L5 (-)	23209	23925
<i>rpl24</i>	50S ribosomal protein L24 (-)	23957	24190
<i>rpl14</i>	50S ribosomal protein L14 (-)	24191	24556
<i>rps17</i>	30S ribosomal protein S17 (-)	24569	24823
<i>rpl29</i>	50S ribosomal protein L29 (-)	24868	25137
<i>rpl16</i>	50S ribosomal protein L16 (-)	25144	25557
<i>rps3</i>	30S ribosomal protein S3 (-)	25609	26253
<i>rpl22</i>	50S ribosomal protein L22 (-)	26295	26642
<i>ORF148</i>	(-)	26691	27137
<i>rps19</i>	30S ribosomal protein S19 (-)	27149	27427
<i>rpl2</i>	50S ribosomal protein L2 (-)	27472	28299
<i>rpl23</i>	50S ribosomal protein L23 (-)	28324	28632
<i>rpl4</i>	50S ribosomal protein L4 (-)	28625	29272
<i>rpl3</i>	50S ribosomal protein L3 (-)	29320	29910
<i>dnaK</i>	Hsp70-type chaperone	30213	32057
<i>groEL</i>	chaperonin, 60 kDa (-)	32125	33711
<i>trnR</i>	tRNA-Arg(acg)	33891	33964
<i>trnQ</i>	tRNA-Gln(uug)	34020	34089
<i>psbW</i>	PSII, protein W, 13 kDa	34326	34673
<i>ycf40</i>	ORF73, homologous to <i>Porphyra</i> ORF71 (-)	34997	35218
<i>trnH</i>	tRNA-His(gug)	35294	35368
<i>rps4</i>	30S ribosomal protein S4	35435	35955
<i>rps16</i>	30S ribosomal protein S16	36238	36477
<i>ycf35</i>	ORF128	36607	36993
<i>psbA</i>	PSII, D1 reaction-center protein (-)	37339	38421
<i>ycf44</i>	ORF382, homologous to <i>Porphyra</i> ORF437 (-)	38642	39790
<i>ycf46</i>	ORF497, homologous to <i>Porphyra</i> ORF491 (-)	39799	41292
<i>rpl34</i>	50S ribosomal protein L34 (-)	41404	41550
<i>secA</i>	preprotein-translocase subunit $\alpha$ (-)	41583	44249
<i>rpl27</i>	50S ribosomal protein L27 (-)	44272	44523
<i>rpl21</i>	50S ribosomal protein L21 (-)	44546	44863
<i>ycf30</i>	former <i>trnE</i> ( <i>rbcR</i> homolog) (-)	45509	46438
<i>trnL</i>	tRNA-Leu(uag) (-)	46484	46564
	<i>end IRb</i>	46634	
<i>rpl32'</i>	50S ribosomal protein L32' (-)	46632	46796
<i>ycf32</i>	ORF36 (-)	46931	47041
<i>rrn5</i>	5S ribosomal RNA (-)	47296	47421
<i>rrn23</i>	23S ribosomal RNA (-)	47493	50383
<i>trnA</i>	tRNA-Ala(ugc) (-)	50449	50521
<i>trnI</i>	tRNA-Ile(gau) (-)	50524	50598
<i>rrn16</i>	16S ribosomal RNA (-)	50665	52149
<i>ORF355</i>	(-)	52814	53881
<i>trnP</i>	tRNA-Pro(ugg) (-)	54019	54092
	<i>start IRb</i>		54358
<i>acp</i>	acyl carrier protein (-)	54453	54695
<i>ycf45</i>	ORF455, homologous to <i>Porphyra</i> ORF565 (-)	54953	56320
<i>rpl20</i>	50S ribosomal protein L20 (-)	56561	56905

<i>rpl35</i>	50S ribosomal protein L35 (-)	56915	57109
<i>ycf42</i>	ORF204, homologous to <i>Porphyra</i> ORF199 (-)	57112	57726
<i>psaE</i>	PSI, subunit IV (-)	57835	58050
<i>ycf25</i>	ORF 644, homologous to <i>E. coli</i> cell-division protein FtsH	58339	60273
<i>rps14</i>	30S ribosomal protein S14	60428	60730
<i>psaM</i>	PSI, protein M	60884	60976
<i>chlI</i>	chlorophyll biosynthesis, probable magnesium-chelatase subunit	61054	62115
<i>ycf47</i>	ORF74, homologous to <i>Porphyra</i> ORF71	62143	62367
<i>trnM</i>	tRNA-Met(cau)	62586	62659
<i>psaD</i>	PSI, ferredoxin-binding protein II	62744	63163
<i>trnS</i>	tRNA-Ser(uga)	63199	63286
<i>petB</i>	cytochrome b6	63341	63988
<i>petD</i>	cytochrome b6/f complex, subunit IV	64035	64517
<i>trnR</i>	tRNA-Arg(ucu) (-)	64774	64845
<i>trnD</i>	tRNA-Asp(guc) (-)	65093	65167
<i>trnS</i>	tRNA-Ser(gcu) (-)	65253	65340
<i>trnfM</i>	tRNA-fMet(cau) (-)	65380	65452
<i>ycf33</i>	ORF64	65614	65808
<i>ORF29a</i>	(A+T)-rich (-)	65920	66009
<i>ORF41</i>	(A+T)-rich (-)	66057	66182
<i>trnY</i>	tRNA-Tyr(gua) (-)	66350	66431
<i>trnV</i>	tRNA-Val(uac)	66555	66626
<i>trnT</i>	tRNA-Thr(ugu)	66647	66717
<i>rbcS</i>	Rubisco, small subunit (-)	66864	67283
<i>rbcL</i>	Rubisco, large subunit (-)	67323	68795
<i>ycf24</i>	ORF486	69086	70546
<i>ycf16</i>	ORF251	70546	71301
<i>atpI</i>	ATP synthase CF <sub>0</sub> subunit IV	71429	72157
<i>atpH</i>	ATP synthase CF <sub>0</sub> subunit III	72227	72475
<i>atpG</i>	ATP synthase CF <sub>0</sub> subunit II	72587	73057
<i>atpF</i>	ATP synthase CF <sub>0</sub> subunit I	73121	73660
<i>atpD</i>	ATP synthase CF <sub>1</sub> subunit $\delta$	73657	74220
<i>atpA</i>	ATP synthase CF <sub>1</sub> subunit $\alpha$	74268	75779
<i>psbB</i>	PSII, CP47 chlorophyll apoprotein	76048	77577
<i>psbT</i>	PSII, protein T, 3 kDa( <i>ycf8</i> )	77629	77727
<i>psbN</i>	PSII, protein N (-)	77752	77883
<i>psbH</i>	PSII, phosphoprotein, 10 kDa	77966	78169
<i>ycf6</i>	ORF29	78255	78344
<i>ycf31</i>	ORF42	78384	78512
<i>psaJ</i>	PSI, subunit IX, 5 kDa (-)	78575	78700
<i>psaF</i>	PSI, subunit III, plastocyanin-binding (-)	78732	79289
<i>psbI</i>	PSII, protein I, 4.8 kDa (-)	79654	79770
<i>ycf41</i>	ORF113, homologous to <i>Porphyra</i> ORF111 (-)	79855	80196
<i>ycf39</i>	ORF319 (-)	80212	81171
<i>cfxQ</i>	involved in Rubisco-expression (-)	81201	82076
<i>psaL</i>	PSI, subunit XI (-)	82171	82623
<i>ycf7</i>	ORF31	82800	82895

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<i>ycf4</i>	ORF181	82917	83462
<i>trnG</i>	tRNA-Gly(ucc)	83508	83578
<i>psbE</i>	cytochrome b559 $\alpha$ -chain	83634	83888
<i>psbF</i>	cytochrome b559 $\beta$ -chain	83901	84032
<i>psbL</i>	PSII, protein L	84068	84184
<i>psbJ</i>	PSII, protein J	84209	84328
<i>ORF26a</i>	(A+T)-rich (-)	84426	84506
<i>ORF380</i>		84538	85680
<i>ORF25</i>	(A+T)-rich (-)	85745	85822
<i>trnE</i>	tRNA-Glu(uuc) (-)	85882	85953
<i>trnG</i>	tRNA-Gly(gcc) (-)	85995	86066
<i>ycf9</i>	ORF61 (-)	86186	86371
<i>ycf12</i>	ORF34 (-)	86419	86523
<i>trnK</i>	tRNA-Lys(uuu)	86730	86801
<i>ORF44</i>	(A+T)-rich	86932	87066
<i>psbC</i>	PSII, CP43 chlorophyll apoprotein (-)	87163	88578
<i>psbD</i>	PSII, D2 reaction-center protein (-)	88526	89581
<i>psaI</i>	PSI, subunit VIII (-)	89824	89940
<i>psbK</i>	PSII, protein K	90146	90280
<i>petG</i>	cytochrome b6/f complex, subunit V	90417	90530
<i>rpl12</i>	50S ribosomal protein L12 (-)	90778	91161
<i>rpl1</i>	50S ribosomal protein L1 (-)	91234	91926
<i>rpl11</i>	50S ribosomal protein L11 (-)	91949	92374
<i>ORF27</i>	(A+T)-rich (-)	92456	92539
<i>trnW</i>	tRNA-Trp(cca) (-)	92660	92732
<i>dnaB</i>	DNA-replication helicase	92815	94182
<i>trnF</i>	tRNA-Phe(gaa) (-)	94184	94256
<i>psbX</i>	PSII, protein X, 4.1 kDa	94593	94709
<i>ORF99</i>		94783	95082
<i>psbV</i>	PSII, cytochrome c550 ( <i>petK</i> )	95139	95630
<i>ORF29b</i>	(A+T)-rich (-)	95659	95748
<i>rpl19</i>	50S ribosomal protein L19	96389	96751
<i>petF</i>	ferredoxin	96895	97194
<i>petA</i>	apocytochrome f (-)	97278	98222
<i>ycf43</i>	ORF263, homologous to <i>Porphyra</i> ORF254 (-)	98256	99047
<i>atpE</i>	ATP synthase CF <sub>1</sub> subunit $\epsilon$ (-)	99151	99552
<i>atpB</i>	ATP synthase CF <sub>1</sub> subunit $\beta$ (-)	99566	100993
<i>ycf3</i>	ORF179 (-)	101162	101701
<i>rps18</i>	30S ribosomal protein S18 (-)	101792	102010
<i>rpl33</i>	50S ribosomal protein L33 (-)	102014	102208
<i>rps20</i>	30S ribosomal protein S20	102420	102701
<i>rpoB</i>	RNA polymerase $\beta$ -chain	102865	107004
<i>rpoC1</i>	RNA polymerase $\beta'$ -chain	107028	109559
<i>rpoC2</i>	RNA polymerase $\beta''$ -chain	109599	114044
<i>ORF26b</i>	(A+T)-rich	114058	114138
<i>rps2</i>	30S ribosomal protein S2	114159	114848
<i>psaB</i>	PSI, P700 apoprotein A2 (-)	114970	117171
<i>psaA</i>	PSI, P700 apoprotein A1 (-)	117351	119609

used, with a preference for TAA. Four genes start with GTG instead of ATG (*rps3*, *rps13*, *rpl23*, *rbcS*).

Identified genes are named according to Hallick and Bottomley (1983) and Hallick (1989). Open reading frames shared by homologous sequences of other chloroplast genomes are designated as *ycf* (Hallick and Bairoch, 1994), genes for tRNAs are labelled using the one-letter code of their respective amino acids. Open reading frames unique to *Odontella* are designated as ORF, followed by the number of encoded amino acid residues. Genes shared with the *Porphyra purpurea* chloroplast genome and the cyanelle genome of *Cyanophora paradoxa*, but unknown from land-plant chloroplast genomes, include *acp*, *atpD*, *atpG*, *dnaK*, *groEL*, *petF*, *psaE*, *psaF*, *psbV*, *psbW*, *psbX*, *rbcS*, *tufA*, *secY*, and *ycf16*, -24, -30, -31, -32, -33, -35, -39. In addition, several genes for ribosomal subunits (*rpl1*, -3, -6, -11, -12, -18, -34, -35, and *rps5*, -6, -10, -13, -17, 20) appear to be unique among plastid genomes of non-green algae. Whereas eight open reading frames (*ycf40-47*) have counterparts in the *Porphyra* chloroplast genome, none of these ORFs were found in the cyanelle genome.

A detailed description of the sequence and its phylogenetic implications will appear elsewhere.

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## Plastid Genomes of Three Non-Green Algae Are Sequenced

The total sequences of the chloroplast genomes of *Marchantia polymorpha* (Ohyama et al., 1986) and *Nicotiana tabacum* (Shinozaki et al., 1986) were reported just nine years ago (see box borrowed from Stirewalt et al., 1995). Apart from the excitement caused by having complete sequences of the largest genomes to that time, these historic discoveries shifted our focus from the question, *What genes are present?* to *How is their expression regulated?* The sequencing of plastid genomes of liverwort and tobacco were followed by those of rice (Hiratsuka et al., 1989), black pine (Wakasugi et al., 1994), *Epifagus virginiana* (Wolfe et al., 1992), *Euglena gracilis* (Hallick et al., 1993), *Zea mays* (Maier et al., 1995), and now *Chlorella ellipsoidea* (M. Sugiura, personal communication).

The accumulated data permit us to make detailed comparisons among plastid genomes: The similarities between vascular and non-vascular plants and between dicots and monocots are far more abundant than are the differences.

- The numbers and kinds of genes differ only slightly.
- Many of the unidentified open reading frames are conserved.
- The occurrence of gene clusters and transcription units are highly conserved.
- The plastid genome of *Epifagus virginiana* has lost all genes encoding photosynthetic components, as one might expect of a non-photosynthetic parasite, but all genes encoding rRNAs and many genes encoding tRNAs are still present and remain functional.

Considering that euglenids have been separated for a billion years from the line that produced higher plants, we expect and find the greatest

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Note: Following in the path of *Arabidupdate*, *OrganelleIntel* is a new feature of the PMBR, and is devoted to new developments in the molecular biology of plant organelles. Suggestions and contributions should be directed to [pmbn@mbcl.rutgers.edu](mailto:pmbn@mbcl.rutgers.edu).

differences in the chloroplast genome of *E. gracilis*. But the differences between the plastid genome of *E. gracilis* and those of higher plants are minor compared to the similarities: genes encoding ribosomal nucleic acids in the *Euglena* plastid are organized in direct rather than inverted repeats, and introns are uncharacteristically abundant in protein-encoding genes.

We now have the sequences of the plastid genomes of three non-green algae: the Glaucocystophyte *Cyanophora paradoxa* (Stirewalt et al., 1995), the red alga *Porphyra purpurea* (Reith & Munholland, 1995), and the diatom *Odontella sinensis* (Kowallik et al., 1995). Gene maps and brief descriptions of each of these genomes appear in the *Genetics Resources* section of this issue. Unlike the plastids of *E. gracilis*, (which may have originated as an endosymbiotic green alga), these plastids are very, very different from the plastids of green algae, and their genomes are correspondingly surprising. Those of us who had hoped for evidence of independent modes of evolution from ancient symbiotic events are not disappointed. Information for protein transport, for example, is encoded within the plastid genome of *C. paradoxa*; localization of fatty acid synthesis in the plastids of higher plants is reflected in the plastid genome of *P. purpurea* by the occurrence of genes encoding enzymes of fatty acid synthesis. Additionally, the *P. purpurea* plastid contains twice as many genes as do plastids of higher plants.

To help us browse among the new gene maps, Michael Reith has constructed a table of genes and gene products (Table I) that occur in these plastid genomes but are absent from plastids of higher plants and *E. gracilis*.

### Totally Sequenced Plastid Genomes

#### Vascular plants

*Epifagus virginiana* (a non-green plant that parasitizes the roots of beech)  
*Marchantia polymorpha* (a liverwort)  
*Nicotiana tabacum* (tobacco)  
*Oryza sativa* (rice)  
*Pinus thunbergii* (black pine)  
*Zea mays* (maize)

#### Algae

*Chlorella ellipsoidea* (a green alga)  
*Cyanophora paradoxa* (an alga with an enigmatic phylogeny)  
*Euglena gracilis* (a nominally green alga with a distinct phylogeny)  
*Odontella sinensis* (a brown alga)  
*Porphyra purpurea* (a red alga)

## The Search Continues

The story is not over. Green algae, euglenids, red algae, brown algae, and the taxonomically singular Glaucocystophyte are only five among many algal phyla that are equally dissimilar: the more we learn about algae, the more we recognize their diversity.

Dinoflagellates should be high on the list of candidates for sequencing; the lack of histones in their nuclear chromosomes already sets them apart from all other eukaryotes. The plastids of some dinoflagellates contain unique light-harvesting complexes, such as peridinin; some plastids appear to be like those of red algae, while still others are pigmented like cyanobacteria.

The plastids of certain cryptomonads and chlorarachniids occur within a larger membrane system that also contains a nucleomorph, which can contain several hundred kb of DNA (Eschbach et al., 1991) and whose genes encode 70 S and 80 S ribosomes (McFadden et al., 1994).

Chloromonads are thought to have departed from the ancestors of green algae very early; their plastid genomes could provide clues to the evolution of chlorophyll *b*-containing lines.

Within the green algal line are the Dasycladales, which include *Acetabularia mediterranea*, a 10-cm cell that contains a single nucleus. Before the tragic death of Hans Schweiger, his group had assembled clear evidence of genes in *A. mediterranea* that were unknown in plastids of higher plants. Tymms & Schweiger (1985) reported that its plastid genome appeared "significantly larger than 400 kb." Considering that the genome of the bacterial parasite *Myxoplasma genitalia* has only 580 kb, one could imagine the plastid of *A. mediterranea* to be nearly self-sufficient.

What can we expect to learn from further investigation of plastid diversity? Will additional patterns of genes retained in plastids offer more solid clues concerning how genes were or were not lost from the hypothetical ancient symbiont(s); or will the evolution of modern plastids be seen as a totally random process? Can we reconstruct discontinued pathways within plastids? Could we engineer more efficient plastids in higher plants with genes borrowed from algae?

**Acknowledgments:** The simultaneous presentation of the three articles on plastid genomes of non-green algae was organized by Hans Bohnert. Stimulating discussions on algae and algal plastids with Hans Bohnert, Michael Reith, and Richard Triemer provided the basis for raising these issues. Any mistakes are ours.

—Ellen M. Reardon and C. A. Price



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**Table 1. Genes and gene products in plastid genomes of non-green algae.** The genes listed were identified in the plastid genomes of *Cyanophora paradoxa*, *Odontella sinensis*, or *Porphyra purpurea*. The list does not include genes that commonly occur in plastid genomes of higher plants. The gene symbols are drawn in part from bacterial gene nomenclature; *ycf* is a system of temporary gene designations assigned to conserved reading frames in chloroplast or plastid genomes (Hallick & Bairoch, 1994). Several of the gene symbols have not yet been approved by the Commission on Plant Gene Nomenclature (<http://probe.nalusda.gov:8300/cgi-bin/browse/mendel>). The table was prepared by Michael Reith in consultation with Hans Bohnert and Klaus Kowallik. RF, reading frame; *C. paradoxa* = *Cyanophora paradoxa*; *O. sinensis* = *Odontella sinensis*; *P. purpurea* = *Porphyra purpurea*.

Gene	Gene Product
<i>accA</i>	acetyl-CoA carboxylase carboxytransferase, $\alpha$ subunit
<i>accB</i>	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit
<i>accD</i>	acetyl-CoA carboxylase carboxytransferase $\beta$ subunit
<i>acpP</i>	acyl carrier protein
<i>apcA</i>	allophycocyanin $\alpha$ subunit
<i>apcB</i>	allophycocyanin $\beta$ subunit
<i>apcD</i>	allophycocyanin $\gamma$ subunit
<i>apcE</i>	phycobilisome core linker polypeptide
<i>apcF</i>	allophycocyanin B18 subunit
<i>argB</i>	acetylglutamate kinase
<i>atpD</i>	ATP synthase CF <sub>1</sub> $\delta$ -subunit
<i>atpG</i>	ATP synthase CF <sub>1</sub> $\gamma$ -subunit
<i>carA</i>	carbamoyl phosphate synthase small subunit
<i>chlB</i>	protochlorophyllide reductase chlB chain
<i>chlI</i>	magnesium chelatase subunit
<i>chlL</i>	protochlorophyllide reductase iron-sulfur ATP-binding protein
<i>chlN</i>	protochlorophyllide reductase ChlN chain
<i>clpC</i>	clp protease ATP-binding subunit
<i>cpcA</i>	phycocyanin $\alpha$ subunit
<i>cpcB</i>	phycocyanin $\beta$ subunit
<i>cpcG</i>	phycobilisome rod-core linker polypeptide
<i>cpeA</i>	phycoerythrin $\alpha$ subunit
<i>cpeB</i>	phycoerythrin $\beta$ subunit
<i>crtE</i>	geranylgeranyl pyrophosphate synthase ( <i>Ggps1</i> )
<i>dnaB</i>	replication helicase subunit
<i>dnaK</i>	hsp70-type chaperone
<i>fabH</i>	$\beta$ -ketoacyl-acyl carrier protein synthase III
<i>frbB</i>	ferredoxin-thioredoxin reductase $\beta$ subunit
<i>ftsW</i>	putative cell (organelle) division protein
<i>glnB</i>	nitrogen regulatory protein PII
<i>gltB</i>	glutamate synthase (GOGAT)
<i>groEL</i>	60-kDa chaperonin

<i>groES</i>	10-kDa chaperonin
<i>hemA</i>	5-aminolevulinic acid synthase
<i>hisH</i>	histidinol-phosphate aminotransferase
<i>hisP</i>	histidine transport ATP-binding protein
<i>ilvB</i>	acetohydroxyacid synthase large subunit
<i>ilvH</i>	acetohydroxyacid synthase small subunit
<i>infB</i>	initiation factor 2
<i>infC</i>	initiation factor 3
<i>nadA</i>	quinolinate synthetase
<i>odpA</i>	pyruvate dehydrogenase E <sub>1</sub> component, $\alpha$ subunit
<i>odpB</i>	pyruvate dehydrogenase E <sub>1</sub> component, $\beta$ subunit
<i>pbsA</i>	heme oxygenase
<i>petJ</i>	cytochrome <i>c</i> <sub>553</sub>
<i>pgmA</i>	phosphoglycerate mutase
<i>preA</i>	prenyl transferase
<i>psaD</i>	photosystem I, ferredoxin-binding protein, subunit II
<i>psaE</i>	photosystem I, subunit IV, 18- to 20 kDa
<i>psaF</i>	plastocyanin-binding protein, subunit III
<i>psaK</i>	photosystem I, PSI-K polypeptide ('P37')
<i>psaL</i>	photosystem I reaction center subunit XI
<i>psbU</i>	9- or 12-kDa protein of oxygen-evolving complex
<i>psbV</i>	cytochrome <i>c</i> <sub>550</sub> (oxygen-evolving complex component)
<i>psbW</i>	photosystem II protein W (13 kDa)
<i>psbX</i>	photosystem II protein X (4.1 kDa)
<i>rbcS</i>	ribulose-bisphosphate carboxylase, small subunit
<i>rne</i>	RNAse E
<i>rnpB</i>	RNA component of RNAse P
<i>rpl1</i> , -3, -4, -5, -6, -9, -11, -12, -13, -18, -19, -24, -27, -28, -29, -31, -34, -35	ribosomal proteins L3-L35
<i>rps1</i> , -5, -6, -9, -10, -13, -17, -20	ribosomal proteins S5-S20
<i>secA</i>	preprotein translocase subunit
<i>secY</i>	preprotein translocase subunit
<i>syfB</i>	phenylalanine tRNA synthetase
<i>syh</i>	histidine tRNA synthetase
<i>thiG</i>	thiG protein, thiamine biosynthesis
<i>trnL(GAG)</i>	transfer RNA leu
<i>trnR(CCU)</i>	transfer RNA arg
<i>trnS(CGA)</i>	transfer RNA ser
<i>trpA</i>	tryptophan synthase $\alpha$ subunit
<i>trpG</i>	anthranilate synthase component II
<i>trxA</i>	thioredoxin
<i>tsf</i>	elongation factor Ts
<i>tufA</i>	elongation factor Tu
<i>ycf16</i>	ABC transporter protein in ATPase operon
<i>ycf17</i>	hypothetical RF 17: ~50 aa—similar to CAB/ELIP/HLIP proteins (in <i>P. purpurea</i> , <i>C. paradoxa</i> , and <i>C. caldarium</i> )
<i>ycf18</i>	hypothetical RF 18: ~58 aa (in <i>P. purpurea</i> and <i>Aglaothamnion</i> sp.)
<i>ycf19</i>	hypothetical RF 19: ~95 aa (in <i>P. purpurea</i> and <i>C. caldarium</i> )

- ycf20 hypothetical RF 20: ~100 aa (in *P. purpurea*, *Aglaothamnion* sp. and *C. caldarium*)
- ycf21 hypothetical RF 21: ~175 aa (in *P. purpurea*, *C. paradoxa*, and *Antithamnion* sp.)
- ycf22 hypothetical RF 22: ~200 aa (in *P. purpurea* and *Antithamnion* sp.)
- ycf23 hypothetical RF 23: ~265 aa (in *P. purpurea*, *C. paradoxa* and *Antithamnion* sp.)
- II ycf24 hypothetical RF 24: ~487 aa (in *P. purpurea*, *C. paradoxa*, *O. sinensis*, others)
- ycf25 hypothetical RF 25: *E. coli* *ftsH* homolog (in *P. purpurea*, *O. sinensis*)
- ycf26 hypothetical RF 26: *E. coli* *envZ* homolog —putative histidine kinase
- ycf27 hypothetical RF 27: *ompR* homolog: putative transcriptional regulatory protein
- ycf28 hypothetical RF 28: *ntcA* homolog: putative transcriptional regulatory protein
- ycf29 hypothetical RF 29: *tctD* homolog: putative transcriptional regulatory protein
- ycf30 hypothetical RF 30: *rbcR* homolog: putative transcriptional regulatory protein
- ycf31 hypothetical RF 31: ~32 aa (in *P. purpurea*, *C. paradoxa*)
- ycf32 hypothetical RF 32: ~37 aa (in *P. purpurea*, *C. paradoxa*)
- ycf33 hypothetical RF 33: ~66 aa (in *P. purpurea*, *C. paradoxa*)
- ycf34 hypothetical RF 34: ~76 aa (in *P. purpurea*, *C. paradoxa*)
- ycf35 hypothetical RF 35: 128 aa (in *P. purpurea*, *C. paradoxa*)
- ycf36 hypothetical RF 36: ~160 aa (in *P. purpurea*, *C. paradoxa*)
- ycf37 hypothetical RF 37: ~172 aa (in *P. purpurea*, *C. paradoxa*)
- ycf38 hypothetical RF 38: 291 aa (in *P. purpurea*, *C. paradoxa*)
- ycf39 hypothetical RF 39: ~320 aa (in *P. purpurea*, *C. paradoxa*)
- ycf40 hypothetical RF 40: ~72 aa (in *P. purpurea*, *O. sinensis*)
- ycf41 hypothetical RF 41: ~112 aa (in *P. purpurea*, *O. sinensis*)
- ycf42 hypothetical RF 42: ~200 aa (in *P. purpurea*, *O. sinensis*)
- ycf43 hypothetical RF 43: ~260 aa (in *P. purpurea*, *O. sinensis*)
- ycf44 hypothetical RF 44: ~400 aa (in *P. purpurea*, *O. sinensis*)
- ycf45 hypothetical RF 45: ~500 aa (in *P. purpurea*, *O. sinensis*)
- ycf46 hypothetical RF 46: ~495 aa (in *P. purpurea*, *O. sinensis*)
- ycf47 hypothetical RF 47: ~72 aa (in *P. purpurea*, *O. sinensis*)

## The Plastid Genome of the Cryptophyte Alga, *Guillardia theta*: Complete Sequence and Conserved Synteny Groups Confirm Its Common Ancestry with Red Algae

Susan E. Douglas, Susanne L. Penny

Institute for Marine Biosciences, 1411 Oxford Street, Halifax, Nova Scotia, Canada B3H 3Z1

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**Abstract.** The plastid genome of the cryptophyte alga *Guillardia theta* (121,524 bp) has been completely sequenced. The genome is 33% G+C and contains a short, nonidentical inverted repeat (4.9 kb) encoding the two rRNA cistrons. The large and small single-copy regions are 96.3 and 15.4 kb, respectively. Forty-six genes encoding proteins for photosynthesis, 5 genes for biosynthetic function, 5 genes involved in replication and division, 30 tRNA genes, 44 ribosomal protein genes (26 large subunit and 18 small subunit), 3 translation factors, 8 genes encoding components of the transcriptional machinery including 3 *ycfs* (hypothetical chloroplast frames), and 26 additional *ycfs* have been identified. There are eight ORFs larger than 50 amino acids, 3 of which have homologues on the plastid genome of the rhodophyte, *Porphyra purpurea* (Reith and Munholland 1995) and/or the *Synechocystis* genome (Kaneko et al. 1996) and can be designated new *ycfs*. Intergenic spacers are very short, no introns have been detected, and several genes overlap, all resulting in a very compact genome. In addition, large clusters of genes (such as those for the ribosomal proteins) are organized into single transcriptional units (Wang et al. 1997), again resulting in an economically organized genome. The cryptophyte plastid genome is almost completely comprised of clusters of genes that are found on the rhodophyte *Porphyra purpurea*, confirming its common ancestry with red algae. Furthermore, recombination events involving both tRNA

genes and the rRNA cistrons appear to have been responsible for the structure of the cryptophyte plastid genome, including the formation of the inverted repeat.

**Key words:** Algae — Cryptophyte — DNA sequence — Endosymbiosis — Evolution — Genome — Plastid

### Introduction

Cryptophytes are an enigmatic group of small biflagellate algae that share pigment characteristics with two distinct algal groups, the rhodophytes (phycobiliproteins) and the chromophytes (chlorophyll *c*). Like chromophytes, they harbour complex plastids surrounded by four membranes, rather than the two surrounding rhodophyte and chlorophyte plastids. However, they differ from chromophytes in possessing a small nucleus-like organelle (the nucleomorph) in the reduced space between the inner and outer plastid membrane pairs (Greenwood et al. 1997).

It has been proposed that organisms containing complex plastids arose by endosymbiosis of a photosynthetic eukaryote and a phagotrophic host with subsequent loss or reduction of eukaryotic features of the endosymbiont, such as the nucleus and cytoplasm (Gillott and Gibbs 1980). The resulting plastids would contain four membranes. Cryptophytes, by possessing vestiges of these eukaryotic features in the form of a nucleomorph and periplastidal space between the inner and the outer plastid membrane pairs, could be thought of as representing an intermediate en route to complex plastids.

Correspondence to: S.E. Douglas; e-mail: susan.douglas@nrc.ca

Ultrastructural data (Gibbs 1981), combined with molecular sequence data (Douglas et al. 1991; Douglas and Murphy 1994), provide strong evidence that cryptophyte algae arose by secondary endosymbiosis of a primitive eukaryotic rhodophyte. Recent phylogenetic analyses have reinforced the sister-group relationship between rhodophytes and nucleomorphs and, also, demonstrated an affiliation between cryptophyte hosts and glaucocystophytes (Bhattacharya and Medlin 1995; Van De Peer et al. 1996).

Plastid gene sequences have been utilized in phylogenetic analyses aimed at determining the relationships among eukaryotic photosynthetic lineages. However, problems encountered with substitutional bias caused by the relatively high A+T content of plastid genes (Lockhart et al. 1992), as well as varying mutational rates of different plastid genes or different sites within genes (Van De Peer et al. 1996) and the possibility of lateral gene transfers (Delwiche and Palmer 1996), have yielded conflicting results that may not reflect the true evolution of these lineages. Comparisons of gene order, on the other hand, offer a means of determining relationships among plastids that are not affected by these phenomena (Kowallik 1989, 1997; Wang et al. 1997).

The recent acquisition of complete genome sequences from the plastids of a number of green (Sugiura 1992; Hallick et al. 1993; Wakasugi et al. 1997) and nongreen photosynthetic eukaryotes (Kowallik et al. 1995; Reith and Munholland 1995; Stirewalt et al. 1995), as well as the cyanobacterium *Synechocystis* PCC6803 (Kaneko et al. 1996), allows new approaches to elucidating evolutionary relationships between algal lineages. In this context, it is of special interest to investigate the coding potential of the plastid of a chlorophyll *c*- and phycobilin-containing alga that may represent an intermediate stage in the evolution of complex plastids that have arisen by secondary endosymbiosis.

Analysis of the content of the *G. theta* plastid genome reveals strong similarities with that from the rhodophyte, *Porphyra purpurea* (Reith and Munholland 1995). Large stretches of DNA are conserved in gene order between the two plastids, although reduced in size in the cryptophyte. In many cases, tRNA genes are at the borders of the conserved stretches and adjacent to genes that have been deleted in the cryptophyte plastid, indicating that rearrangements have arisen through recombination between nonhomologous tRNA genes, as described in rice (Hiratsuka et al. 1989). In addition, recombination between the directly repeated rRNA cistons of the ancestral rhodophyte plastid appears to have resulted in the formation of the inverted repeat of the present-day cryptophyte plastid.

## Materials and Methods

*Guillardia theta*, formerly designated *Cryptomonas* 'D' (Hill and Wetherbee 1990), was cultivated as described (Douglas 1988), and plastid

DNA was isolated by cesium chloride equilibrium centrifugation in the presence of Hoechst 33258 (Douglas 1988). The majority of the chloroplast genome was subcloned into pUC19 (Pharmacia) using a variety of restriction enzymes. A small portion of the genome that could not be cloned into pUC19 due to a lack of appropriate restriction enzyme sites was amplified by PCR using the high accuracy polymerase Pfu (Stratagene) and cloned into the vector pCR2.1 (Invitrogen). At least three clones of each amplification product were sequenced to reduce the possibility of PCR-generated artefacts. Template DNA was prepared using the Nucleobond AX kit (Machery Nagel) and sequencing was performed using an ABI 373A automated sequencer and the AmpliTaqFS dye terminator cycle sequencing ready reaction kit (Perkin Elmer). Specific oligonucleotide primers were used to fill gaps and complete the sequence of both strands. Sequence analysis and contig assembly was performed using Sequencher (Gene Codes, Inc.). Coding regions were identified by BLAST searches of GenBank (Gish and Gates 1993) and automated database searches were performed using MAGPIE (Gaasterland and Sensen 1996). Codon usage (based on known coding sequences including *ycfs*) was calculated using DNA Strider (Marck 1988).

## Results and Discussion

**Genome Organization.** The circular plastid DNA of *Guillardia theta* is 121,524 bp, contains two small (approximately 4.9-kb) rRNA-containing inverted repeats, and encodes 183 genes (including the duplicated rRNA cistron genes) that are equally distributed on both strands (Fig. 1). It is the epitome of compactness (90% is coding sequence), exhibiting short A+T-rich intergenic spacers, no pseudogenes or introns, and four cases of overlapping genes. This is similar to the situation in the rhodophyte *P. purpurea* and the chromophyte *Odontella sinensis* but contrasts with green plants such as rice, where only 68% of the plastid genome is coding sequence (Hiratsuka 1989), the inverted repeats are much larger, and pseudogenes and introns are commonly found (see Sugiura 1992). All except six of the open reading frames (ORFs) have homologs on at least one other plastid genome. The ORFs without clear plastid homologues include *hlpA*, which encodes a histone-like protein (Wang and Liu 1991; Grasser et al. 1997) and appears to be unique to *G. theta*, and ORFs 53, 62, 65, 125, and 252. Other than the ribosomal RNAs, no genes for structural RNAs, such as the RNA component of RNase P (*mpB*) that is present in *Cyanophora paradoxa* (Stirewalt et al. 1995) and *P. purpurea* (Reith and Munholland 1995), have been detected.

Like most plastids, the G+C content is low (33%), and interestingly, identical to that of *P. purpurea* (Reith and Munholland 1995). The codon usage reflects this bias, with codons ending in G and C comprising only 19% of the total. However, the highly expressed genes *psbA* and *rbcL* have a different codon bias that may be a result of selection for increased translation efficiency (Morton 1998). Alternatively, the distinct codon usage of these two genes could reflect their different origin resulting from horizontal gene transfer events. Codon usage of the five unidentified ORFs is similar to that of known coding regions, indicating that they are bona fide reading frames. The ochre termination codon TAA is used in 77% of cases, with amber and opal codons being used 15

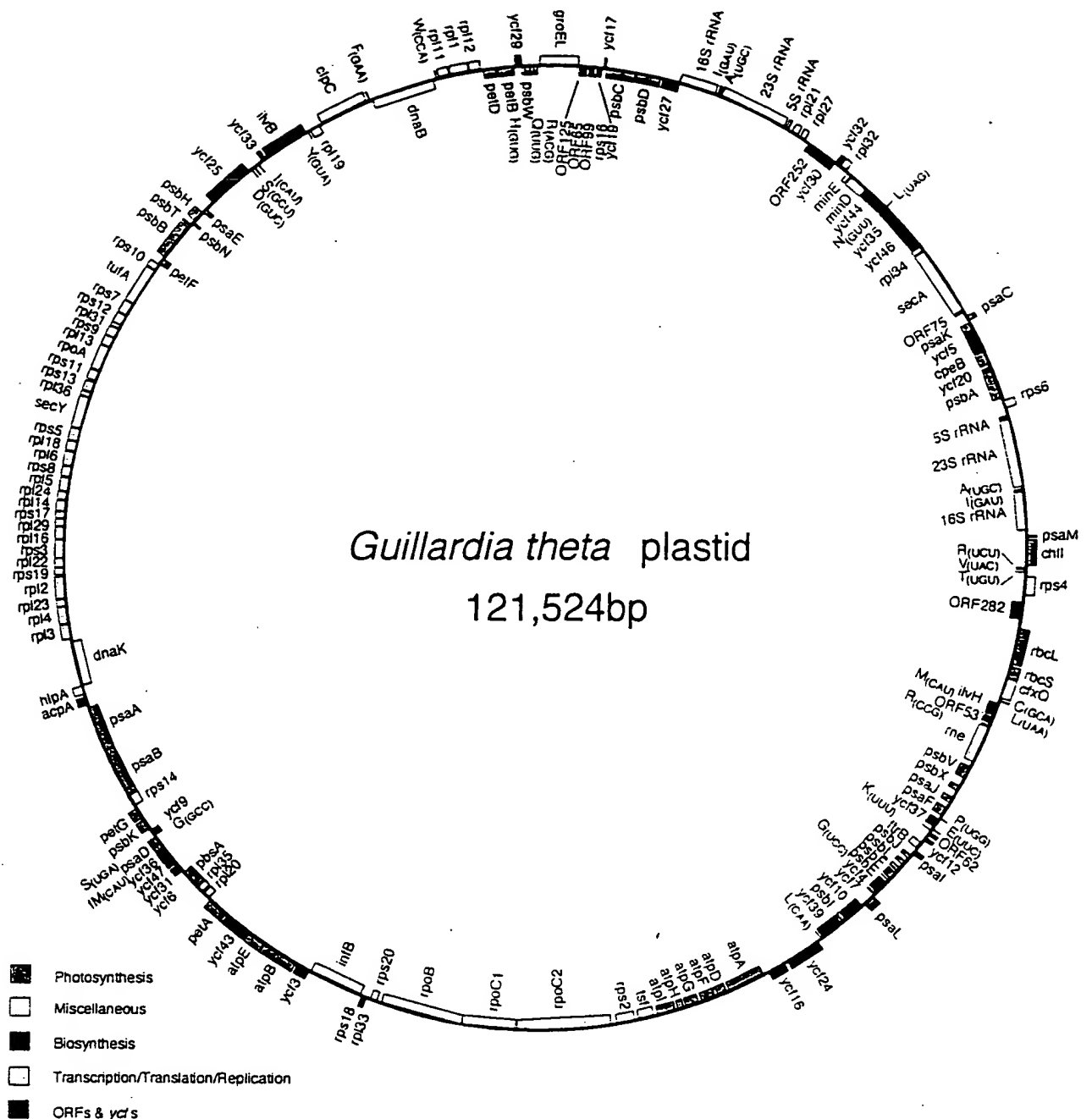


Fig. 1. Gene map of the plastid genome of *G. theta*. Genes transcribed from the plus strand are depicted outside the circle and those from the minus strand inside. Genes are shaded according to function as shown in the key.

and 8%, respectively. ATG is the predominant initiation codon, but GTG is used seven times and TTG once.

**Transcription.** Transcription in plastids is performed by two types of RNA polymerase, a multisubunit eubacteria-like plastic-encoded polymerase and a single-subunit phage T7-like nuclear-encoded polymerase (Allison et al. 1996). Four subunits of the eubacteria-like RNA polymerase (*rpoA*, *rpoB*, *rpoC1*, and *rpoC2*) are encoded on the *G. theta* plastid genome.

There is considerable evidence for polycistronic transcription of genes for related functions, not only from the

presence of gene clusters on plastid genomes, but also from transcription studies (Douglas and Murphy 1994; Löffelhardt et al. 1997; Wang et al. 1997). Putative promoters similar to the canonical prokaryotic-type promoter sequences have been reported upstream of many *G. theta* plastid genes (Douglas et al. 1990; Douglas and Turner 1991; Douglas and Murphy 1994; Wang et al. 1997) and inspection of sequences upstream of polycistronic transcripts known from cyanobacteria and other plastids (see Löffelhardt et al. 1997) have revealed several more putative promoters. In all cases, the -10 TATAAT consensus is present, although the -35 con-

Table 1. Distribution of *ycf*s among photosynthetic lineages<sup>a</sup>

Name	Putative function	Synonym	Gr	C.P.	O.s.	G.t.	P.p.	Syn
<i>ycf1</i>	Hypothetical chloroplast RF1		+					
<i>ycf2</i>	Hypothetical chloroplast RF2	<i>ftsH</i> partially	+					
<i>ycf3</i>	Stable accumulation of PSI complex		+	+	+	+	+	+
<i>ycf4</i>	Stable accumulation of PSI complex		+	+	+	+	+	+
<i>ycf5</i>	Heme attachment to c-type cytochromes	<i>ccsA</i>	+	+	+	+	+	+
<i>ycf6</i>	Hypothetical chloroplast RF6		+	+	+	+	+	+
<i>ycf7</i>	Subunit of cytochrome b6f complex	<i>petl</i>	+	+	+	+	+	+
<i>ycf8</i>	PSII subunit req'd under stress	<i>psbT</i>	+	+	+	+	+	+
<i>ycf9</i>	Hypothetical chloroplast RF9		+	+	+	+	+	+
<i>ycf10</i>	Inorganic carbon uptake	<i>cotA/cemA</i>	+			+	+	+
<i>ycf11</i>	Acetyl-CoA carboxylase beta subunit	<i>accD/zfpA</i>	+				+	+
<i>ycf12</i>	Hypothetical chloroplast RF12		+	+	+	+	+	+
<i>ycf13</i>	Maturase-like protein	<i>matA</i>	+					
<i>ycf14</i>	Hypothetical chloroplast RF14 (intron)	<i>matK</i>	+					
<i>ycf15</i>	Hypothetical chloroplast RF15		+					
<i>ycf16</i>	ABC transporter subunit			+	+	+	+	+
<i>ycf17</i>	Similar to CAB/ELIP/HLIP protein			+		+	+	+
<i>ycf18</i>	Hypothetical chloroplast RF18	<i>nblA</i>					+	+
<i>ycf19</i>	Hypothetical chloroplast RF19					+	+	+
<i>ycf20</i>	Hypothetical chloroplast RF20					+	+	+
<i>ycf21</i>	Hypothetical chloroplast RF21			+			+	+
<i>ycf22</i>	Hypothetical chloroplast RF22						+	+
<i>ycf23</i>	Hypothetical chloroplast RF23			+			+	+
* <i>ycf24</i>	ABC transporter subunit			+	+	+	+	+
<i>ycf25</i>	Homologous to <i>E. coli</i> protein <i>ftsH</i>		+		+	+	+	+
<i>ycf26</i>	<i>envZ</i> homologue putative His kinase	<i>dfr</i>					+	+
<i>ycf27</i>	<i>ompR</i> homologue, putative trp			+	+	+	+	+
<i>ycf28</i>	<i>nucA</i> homologue, putative trp						+	+
<i>ycf29</i>	<i>ictD</i> homologue, putative up			+		+	+	+
<i>ycf30</i>	<i>lysR</i> homologue, putative trp	<i>rbcR</i>		+	+	+	+	+
<i>ycf31</i>	Cytochrome b6f complex subunit	<i>petM</i>		+	+	+	+	+
<i>ycf32</i>	Photosystem II thylakoid protein			+	+	+	+	+
<i>ycf33</i>	Hypothetical chloroplast RF33			+	+	+	+	+
<i>ycf34</i>	Hypothetical chloroplast RF34			+			+	+
<i>ycf35</i>	Hypothetical chloroplast RF35			+	+	+	+	+
<i>ycf36</i>	Hypothetical chloroplast RF36			+		+	+	+
<i>ycf37</i>	Hypothetical chloroplast RF37			+		+	+	+
<i>ycf38</i>	Hypothetical chloroplast RF38			+			+	+
<i>ycf39</i>	Hypothetical chloroplast RF39			+	+	+	+	+
<i>ycf40</i>	Hypothetical chloroplast RF40				+		+	+
<i>ycf41</i>	Hypothetical chloroplast RF41				+		+	+
<i>ycf42</i>	Hypothetical chloroplast RF42	<i>basI</i>			+		+	+
<i>ycf43</i>	Potential integral membrane protein	<i>yigU/ycbT</i>			+	+	+	+
<i>ycf44</i>	c-type holocytochrome formation	<i>ccs</i>			+	+	+	+
<i>ycf45</i>	Hypothetical chloroplast RF45				+		+	+
<i>ycf46</i>	Hypothetical chloroplast RF46				+	+	+	+
<i>ycf47</i>	Hypothetical chloroplast RF47				+	+	+	+
<i>ycf61</i>	Hypothetical chloroplast RF48	ORF75				+	+	+
<i>ycf65</i>	Hypothetical chloroplast RF49	ORF99b				+	+	+
<i>ycf80</i>	Hypothetical chloroplast RF50	ORF282				+	+	

<sup>a</sup> Lineages or their members are abbreviated as follows: green algae and land plants, Gr; *Cyanophora paradoxa*, C.p.; *Odontella sinensis*, O.s.; *Guillardia theta*, G.t.; *Porphyra purpurea*, P.p.; and *Synechocystis* PCC. 6803, Syn. Presence of a *ycf* is indicated by a + symbol. trp, transcriptional regulatory protein.

sensus is sometimes absent. It is possible that a nuclear-encoded polymerase transcribes those genes where canonical prokaryotic-type promoters are absent, although the 10-nucleotide consensus promoter sequence identified from tobacco (Hajdukiewicz et al. 1997) could not be detected.

Plastid gene expression in chloroplasts is regulated mainly at the posttranscriptional level (Danon 1997).

However the presence of three potential genes with significant similarity to transcriptional regulatory proteins (*ycf27*, *ycf29*, *ycf30*) in *G. theta*, *P. purpurea*, *C. paradoxa*, and *O. sinensis* plastid genomes (Table 1) and a gene for an ATP-binding polypeptide involved in the expression of Rubisco (*cfxQ*) in all except *C. paradoxa* (Table 2) indicates that at least some gene expression occurs by transcriptional regulation. Ribonuclease E



Table 2. Distribution of genes among nongreen plastid genomes<sup>a</sup>

Name	<i>C.p.</i>	<i>O.s.</i>	<i>G.t.</i>	<i>P.p.</i>
ATP synthase				
atpA	+	+	+	+
atpB	+	+	+	+
atpD	+	+	+	+
atpE	+	+	+	+
atpF	+	+	+	+
atpG	+	+	+	+
atpH	+	+	+	+
atpI		+	+	+
Photosystem I				
psaA	+	+	+	+
psaB	+	+	+	+
psaC	+	+	+	+
psaD		+	+	+
psaE	+	+	+	+
psaF	+	+	+	+
psaI	+	+	+	+
psaJ	+	+	+	+
psaK			+	+
psaL		+	+	+
psaM	+	+	+	+
Photosystem II				
psbA	+	+	+	+
psbB	+	+	+	+
psbC	+	+	+	+
psbD	+	+	+	+
psbE	+	+	+	+
psbF	+	+	+	+
psbH	+	+	+	+
psbI	+	+	+	+
psbJ	+	+	+	+
psbK	+	+	+	+
psbL	+	+	+	+
psbN	+	+	+	+
psbT(ycf8)	+	+	+	+
psbV	+	+	+	+
psbW	+	+	+	-
psbX	+	+	+	-
Rubisco				
rbcL	<sup>b</sup>	+	+	+
rbcS	<sup>b</sup>	+	+	-
Phycobiliproteins				
apcA	+			+
apcB	+			+
apcD	+			-
apcE	+			-
apcF	+			+
cpcA	+			-
cpcB	+			+
cpcG	+			-
cpeA				-
cpeB			+	+
Electron transfer				
petA	+	+	+	+
petB	+	+	+	-
petD	+	+	+	+
petF	+	+	+	+
petG	+	+	+	+
petL (ycf7)	+	+	+	+
petM (ycf31)	+	+	+	-
ftbB			+	+
Miscellaneous				
clpC		+	+	+
dnaB		+	+	+

Table 2. Continued

Name	<i>C.p.</i>	<i>O.s.</i>	<i>G.t.</i>	<i>P.p.</i>
dnaK	+	+	+	+
groEL	+	+	+	+
secA		+	+	+
secY	+	+	+	+
Ribosomal proteins				
rpl1	+	+	+	+
rpl2	+	+	+	+
rpl3	+	+	+	+
rpl4		+	+	+
rpl5	+	+	+	+
rpl6	+	+	+	+
rpl9				+
rpl11	+	+	+	+
rpl12	+	+	+	+
rpl13		+	+	+
rpl14	+	+	+	+
rpl16	+	+	+	+
rpl18	+	+	+	+
rpl19	+	+	+	+
rpl20	+	+	+	+
rpl21	+	+	+	+
rpl22	+	+	+	+
rpl23		+	+	+
rpl24		+	+	+
rpl27		+	+	+
rpl28	+			+
rpl29		+	+	+
rpl31		+	+	+
rpl32		+	+	+
rpl33	+	+	+	+
rpl34	+	+	+	+
rpl35	+	+	+	+
rpl36	+	+	+	+
rps1				+
rps2	+	+	+	+
rps3	+	+	+	+
rps4	+	+	+	+
rps5	+	+	+	+
rps6	+	+	+	+
rps7	+	+	+	+
rps8	+	+	+	+
rps9	+	+	+	+
rps10	+	+	+	+
rps11	+	+	+	+
rps12	+	+	+	+
rps13	+	+	+	+
rps14	+	+	+	+
rps16	+	+	+	+
rps17	+	+	+	+
rps18	+	+	+	-
rps19	+	+	+	+
rps20	+	+	+	+
Transcription/RNA processing				
cfxQ		+	+	-
rne			+	-
mpB	+			-
rpoA	+	+	+	+
rpoB	+	+	+	+
rpoC1	+	+	+	-
rpoC2	+	+	+	-
Translation				
infB			+	-

Table 2. Continued

Name	<i>C.p.</i>	<i>O.s.</i>	<i>G.t.</i>	<i>P.p.</i>
<i>tsf</i>			+	+
<i>tufA</i>	+	+	+	+
Biosynthesis				
<i>accA</i>	+	+	+	+
<i>chlB</i>	+			+
<i>chlI</i>	+	+	+	+
<i>chlL</i>	+			+
<i>ChlN</i>	+			+
<i>ilvB</i>			+	+
<i>ilvH</i>			+	+
<i>pbsA</i>			+	+
<i>preA</i>	+			+
<i>trpC</i>	+			+

<sup>a</sup> Except for ribosomal proteins, genes unique to a single genome are not shown. Abbreviations are as in Table 1.

<sup>b</sup> The rubisco genes of the *C. paradoxa* plastid are not homologous to those of the other plastids.

(*rne*), also encoded on the plastid genome of *G. theta*, may participate in posttranscriptional degradation of mRNAs.

**Translation.** Many components of the translational apparatus are present, including the 3 rRNA molecules, 1 initiation factor (*infB*), two elongation factors (*tsf* and *tufA*), 26 genes for 50S ribosomal subunit proteins, and 18 genes for 30S ribosomal subunit proteins. Most of these ribosomal protein genes are found in a large cluster which is conserved to different degrees in different photosynthetic lineages and has been found to be a useful character for phylogenetic reconstruction (Sugita et al. 1997; Wang et al. 1997). In addition, several other highly conserved ribosomal protein gene clusters are present (*rpl11/1/12*, *rpl33/rps18*, *rpl20/35*, and *rpl21/27*). Thirty tRNAs are present, two of which (*trnI* and *trnA*) are duplicated in the inverted repeats. This suite of tRNAs allows the decoding of all 61 sense codons.

**Photosynthesis.** All of the components of the ATP synthase with the exception of *atpC*, which was transferred to the nucleus very early in the evolution of plastids (Pancic et al. 1992; Kowallik 1997), are present on the *G. theta* plastid genome. As in both *P. purpurea* and *O. sinensis*, there is an overlap of four nucleotides between the *atpF* and the *atpD* genes. Interestingly, these two genes overlap by a single nucleotide in the cyanobacterium *Synechococcus* PCC 6301 (Cozens and Walker 1987).

Seven components of the electron transfer chain are also found (*petA*, *B*, *D*, *F*, and *G*), including the recently identified *petL* [formerly designated *ycf7* (Naithani et al. 1997)] (Table 1) and *petM* [formerly designated *ycf31* (de Vitry et al. 1996)] (Table 1). With the exception of *psbM*, which has been identified only on the *C. paradoxa* plastid genome, the complete suite of 28 photosystem I and II genes is present on the *G. theta* plastid genome. Also present is the beta subunit of ferredoxin thioreduc-

tase (*frbB*), which participates in electron transfer and regulates several photosynthetic enzymes. In addition, genes for both subunits of Rubisco (*rbcL* and *rbcS*), the beta subunit of phycoerythrin (*cpeB*), and two genes involved in chlorophyll biosynthesis—a magnesium chelatase (*chlI*) and heme oxygenase (*pbsA*)—are found on the *G. theta* plastid genome. *ycf17*, which encodes a protein similar to members of the CAB/ELIP/HLIP family, is also present.

**Replication and Cell Division.** Although plastids lack histones, there is evidence for chromatin-associated proteins (see Grasser et al. 1997). One such protein, encoded by *hlpA* (Wang and Liu 1991), is thought to perform an architectural role in the plastid nucleoid (Grasser et al. 1997). In addition, *dnaB*, encoding a DNA helicase, and three other genes that participate in cell division (*minD*, *minE*, *fisH*) that were recently reported from the plastid genome of the green alga *Chlorella vulgaris* C-27 (Wakasugi et al. 1997) have been identified on the *G. theta* plastid genome. It is interesting that *minD* and *minE* have not been identified in any other nongreen algae and *hlpA* is unique among all sequenced plastids. This may indicate that the cryptophyte endosymbiont represents a primitive stage in the evolution of the nongreen lineages, just as *C. vulgaris* represents a primitive stage in the green lineage.

**Miscellaneous Functions.** A number of genes involved in protein metabolism or transport are encoded on the plastid genome of *G. theta*. These include *secA* and *secY*, which are components of the sec protein translocation system, *groEL* and *dnaK* (chaperonin subunits), and *clpC* (the ATP binding subunit of the Clp protease).

**Conserved Reading Frames (ycfs).** Of the 47 reading frames that are conserved between at least two plastid genomes and are designated by the Commission on Plant Gene Nomenclature (Hallick and Bairoch 1994) as *ycfs* (hypothetical chloroplast frames), 29 have been identified on the plastid of *G. theta*. The distribution of these among various plastid groups and the functions of those that are known are listed in Table 1. In addition, *G. theta* ORFs 76, 99, and 282 are homologous to ORFs 75, 99b, and 450 of *P. purpurea* and are now designated *ycfs* 61, 65, and 80, respectively (Stoebe, personal communication).

**A Conserved Intein.** The *dnaB* genes from both *G. theta* and *P. purpurea* contain an additional stretch of protein-encoding sequence that is spliced out of the mature polypeptide, much as an intron is spliced out of mature mRNA. Inteins in the *dnaB* gene are relatively rare, being found only in two eubacteria, *Synechocystis* PCC6803 and *Rhodothermus marinus* (Liu and Hu 1997). The only other inteins known to occur in plastids are in the *clpP* genes of *Chlamydomonas reinhardtii* and *C. eugametos* (Huang et al. 1994). The *G. theta dnaB* intein is 160 amino acids long, whereas the *P. purpurea*

intein is 150 amino acids long. In both organisms, the codon usage of the intein is very similar to that of the exons, indicating that it is quite ancient and the codon usage has become homogenized over time.

**The Inverted Repeat.** Examination of the regions flanking the cryptophyte *rrnB* cistron shows that it contains genes from the upstream region of *rrnB* (*trnV*, *trnR*, *chlI*, and *psaM*) and the downstream region of *rrnA* (*rps6*) of *P. purpurea* (Fig. 2). The reciprocal arrangement (involving 36.9 kb of sequence upstream of the *P. purpurea* *rrnA* cistron) is evident in the cryptophyte *rrnA* cistron. It is highly likely that the inverted repeat structure of the cryptophyte plastid has resulted from a reciprocal recombination event within the rRNA cistron.

The inverted repeats of *G. theta* are not identical in sequence. There is one transition substitution in the SSU rRNA gene, two in the tRNA<sup>Ala</sup>-LSU rRNA intergenic-spacer, three in the LSU rRNA gene, one in the LSU rRNA-5S rRNA intergenic spacer, and two in the 5S rRNA gene. None of the substitutions affect secondary structure of the mature rRNA molecules. The regions upstream of the 16S rRNA genes are well conserved (86% similar) for 111 bp, presumably to preserve the region surrounding the promoter. However sequence similarity stops immediately downstream of the 5S rRNA gene. In *P. purpurea*, the coding sequences are much more variable than in *G. theta*, with 41 of 4820 positions differing (Reith and Munholland 1995), and the flanking sequences diverge within seven nucleotides of the 16S rRNA and within two of the 5S rRNA (Reith and Munholland 1993). This implies that the copy-correction mechanism that ensures identity of repeats in land plant chloroplasts, but is apparently absent in *P. purpurea*, may be only partially developed in *G. theta*. Similarly, the expansion of the inverted repeat by gene conversion, which has occurred to differing extents in other lineages, may have occurred to a limited extent in *G. theta* since the region upstream of the SSU rRNA is conserved for a short distance.

**Synteny Groups.** The entire plastid genome of *G. theta* is comprised of synteny groups that are present in *P. purpurea* (Fig. 3; junctions marked by arrowheads). Three of these synteny groups are very large (two are over 30 kb and one is 17 kb). In all cases, the gene order and transcriptional orientation are conserved, but some genes present on the plastid genome of *P. purpurea* (usually those involved in biosynthesis, phycobiliprotein synthesis or ORFs of unknown function) have been deleted from the plastid genome of *G. theta* (Fig. 4). In fact there are only five genes involved in biosynthesis remaining on the *G. theta* plastid genome (*ilvB*, *ilvH*, *pbsA*, *chlI*, and *acpA*), a single subunit of phycoerythrin (*cpeB*), and five ORFs of unknown function.

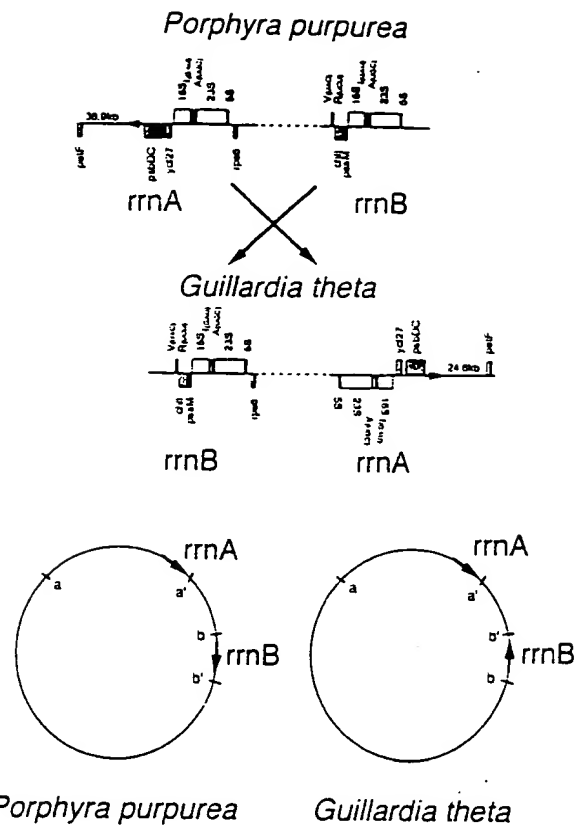


Fig. 2. Recombination between ribosomal RNA cistrons. A reciprocal crossover event between *rrnA* and *rrnB* of *P. purpurea* results in the exchange of flanking sequences and the resulting arrangement seen in *G. theta*. Deletion of several genes has resulted in the reduction in size of the *rrnA* flanking region from 36.9 kb in *P. purpurea* to 24.6 kb in *G. theta*. Borders of *rrnA* and *rrnB* are represented by a/a' and b/b', respectively.

In many cases, tRNA genes are present at the junctions of the synteny groups (asterisks; Fig. 3), suggesting that they may have participated in the deletion of gene sequences, possibly by acting as recognition signals (Hiratsuka et al. 1989). In addition, there are sixteen instances where tRNA genes are also found adjacent to *P. purpurea* genes that have been deleted from *G. theta*. Figure 4B shows one such region of the *P. purpurea* genome where three deletions have occurred relative to *G. theta*, all of which are adjacent to tRNA genes.

**Evolutionary Implications.** This is the first plastid genome to be sequenced from a nucleomorph-containing organism and as such it is of interest to compare its coding capacity with that of other algae that have arisen by secondary endosymbiosis but do not contain a nucleomorph. The diatom *O. sinensis* is one such example that has been completely sequenced (Kowallik et al. 1995). Although some synteny groups are shared between *G. theta* and *O. sinensis* (large ribosomal protein, *atpA*, *rpoBC1C2*, *psbBTNH* gene clusters), none are as large or as striking as those shared between *G. theta* and *P. purpurea*. There has been much more rearrangement (Fig. 4), indicating either that a longer period of evolution has

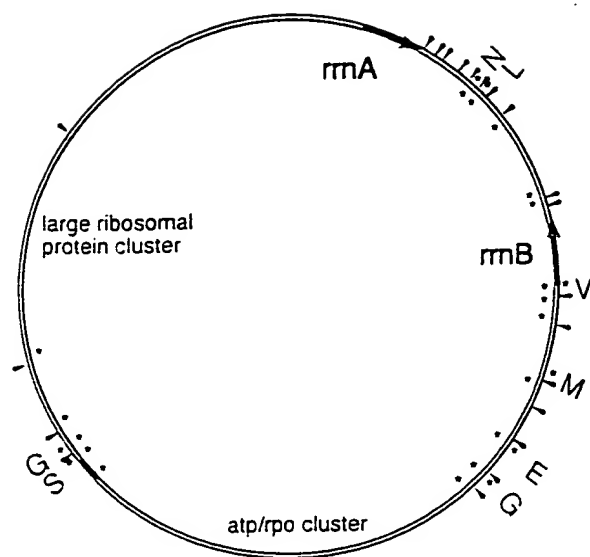


Fig. 3. Synteny groups present on the *G. theta* plastid genome. Junctions between conserved gene clusters are indicated by arrows. Asterisks inside the circle represent tRNA genes present at the junctions on the *P. purpurea* plastid genome and those outside the circle (with letters) represent those at the junctions on the *G. theta* plastid genome. The two rRNA cistrons are represented by shaded arrows and an internal inversion of the cluster *ycf6/31/47/36/rnfMet* within the larger synteny group is indicated by shading.

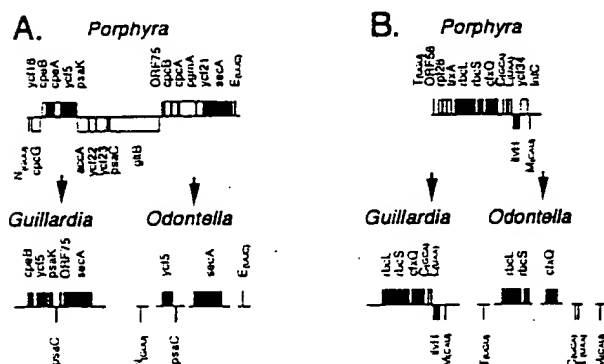


Fig. 4. (A) Gene arrangement between  $tRNA^N$  and  $tRNA^E$  genes of *P. purpurea*, *O. sinensis*, and *G. theta*. (B) Gene arrangement between  $tRNA^T$  and  $tRNA^M$  genes of *P. purpurea*, *O. sinensis*, and *G. theta*. Genes present in both *P. purpurea* and *G. theta* are represented by shaded boxes and those that have been deleted from *G. theta* by empty boxes. Additional genes have been deleted from *O. sinensis*. Genes transcribed from the plus strand are depicted above the line and those from the minus strand below.

passed since the *O. sinensis* endosymbiont was established than for the *G. theta* endosymbiont or that the *O. sinensis* and *G. theta* endosymbionts were different. Alternatively, the presence of genes essential for plastid function in the nucleomorph have stabilized the plastid genome such that rearrangements do not occur to as great an extent in nucleomorph-containing organisms. That possibility is now under investigation although preliminary results indicate that there are very few genes for plastid-localized products in the nucleomorph (McFadden et al. 1997).

With the availability of complete plastid genome sequences, analysis of the distribution of gene clusters has increasingly been used for phylogenetic reconstruction (Kowallik 1997). Of particular interest are clusters that are widely separated on cyanobacterial genomes but appear to have fused subsequent to endosymbiosis and are present in plastid genomes from several lineages. Such arrangements provide very strong evidence for the monophyletic origin of plastids. For such similar organization in the plastid genomes of separate lineages to result from different endosymbionts, an extreme degree of convergent evolution would have to be invoked (Douglas 1994). Well-studied examples that have helped elucidate phylogenetic relationships between algal lineages include the large ribosomal protein cluster (Wang et al. 1997), the rRNA cistrons (Reith and Munholland 1993), the *rpoBC/atpA* cluster (Pancic et al. 1992; Kowallik 1997), and the *psbBTNH* cluster (Douglas 1994).

Three significant features suggest that the ancestor of the *G. theta* plastid closely resembled a rhodophyte plastid like that of *P. purpurea*. First, the conserved synteny groups, which are identical in gene order but reduced in gene content to stretches of the *P. purpurea* plastid genome, give strong evidence for a common ancestry. Second, both *G. theta* and *P. purpurea* contain an intein in their plastid *dnaB* genes. Given the rarity of inteins in plastid genes in general, and *dnaB* genes in particular, this is a significant shared character. Third, the inverted repeat of *G. theta* appears to have arisen by reciprocal recombination from the nonidentical, directly repeated rRNA cistrons of *P. purpurea* (interpreted by the authors as being a primitive feature) (Reith and Munholland 1993). Our results greatly strengthen their suggestions that the ancestral plastid may have had two direct, non-identical rRNA repeats that were either reorganized into the inverted pattern seen in many land plants, glaucophytes, rhodophytes, cryptophytes, and chromophytes or reduced to a single copy in some chlorophytes and rhodophytes.

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*Genetic Resource*

## Nucleotide Sequence of the Cyanelle Genome from *Cyanophora paradoxa*

Veronica L. Stirewalt, Christine B. Michalowski,  
Wolfgang Löffelhardt, Hans J. Bohnert, and Donald A. Bryant

E-mail: dab14@psuvm.psu.edu; bohnert@biosci.arizona.edu  
(VLS, DAB) Department of Biochemistry and Molecular Biology, The  
Pennsylvania State University, University Park, PA, 16802, U.S.A.  
(CBM, HJB) Departments of Biochemistry, Molecular and Cellular Biology,  
and Plant Sciences, The University of Arizona, Tucson, AZ 85721, U.S.A.  
(WL) Institut für Biochemie und Molekulare Zellbiologie und  
Ludwig-Boltzmann-Forschungsstelle für Biochemie, Universität Wien,  
A-1030, Vienna, Austria.

**Key Words:** *Cyanophora paradoxa*, cyanelle, chloroplast, plastid, nucleotide sequence.

**Abstract:** The complete nucleotide sequence of the cyanelle genome of *Cyanophora paradoxa* Pringsheim strain LB 555 was determined (accession number U30821). The circular molecule is 135,599 base pairs in length. The physical map of this DNA molecule is shown along with identified genes and open reading frames.

**C***yanophora paradoxa* (Glaucocystophyceae) is a bi-flagellated protist that contains cyanobacterium-like plastids termed cyanelles. The cyanelles of *Cyanophora paradoxa* are conspicuous because they are surrounded by a lysozyme-sensitive peptidoglycan wall that is typical of those associated with cyanobacteria but that is not found in other plastid types. Thus, this organism has frequently been considered a "living fossil" and a paradigm for the invasion of a eukaryotic cell by a cyanobacterium (for a review, see Löffelhardt and Bohnert, 1994). The complete nucleotide sequence, 135,599 bp, of the cyanelle DNA from the

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Abbreviations: LSC, large single-copy region; SSC, small single-copy region.

unicellular alga *Cyanophora paradoxa* Pringsheim strain LB555 has been determined (GeneBank accession number: U30821). The chromosome (Fig. 1) has a G+C content of 30.4 percent and is characterized by two inverted-repeat segments of 11,285 bp each ( $IR_A$  and  $IR_B$ , respectively), separated by a large single-copy region (LSC) of 94,946 bp and a small single-copy region (SSC) of 18,083 bp. Approximately 192 identified genes and open reading frames (of which ten are duplicated in the inverted repeats) have been identified thus far. Although the genome is smaller than that of tobacco, it encodes about 30 percent more genes than does the tobacco chloroplast genome. Genes encoded in the chloroplast genomes of higher plants but not found in the cyanelle genome include *ndhA-K*, *rps15*, *rpl23*, *infA*, *atpI*, *accD*, and *matK*.

To catalog genes and reading frames on the cyanelle DNA, the molecule has arbitrarily been linearized at the beginning of the inverted repeat 5' to the end of one of the 16S rRNA genes. Counting proceeds through  $IR_A$ , LSC,  $IR_B$ , and SSC (Fig. 1). Identified genes are given names as recommended (Hallick and Bottomley, 1983; Hallick, 1989); unknown reading frames larger than 25 codons are labeled "ORF" followed by the number of codons. Unknown reading frames that have been found in other plastid DNAs are labeled *ycf* (Hallick and Bairoch, 1993). Genes for tRNAs are identified by the amino acid with which they are charged in the single-letter code.

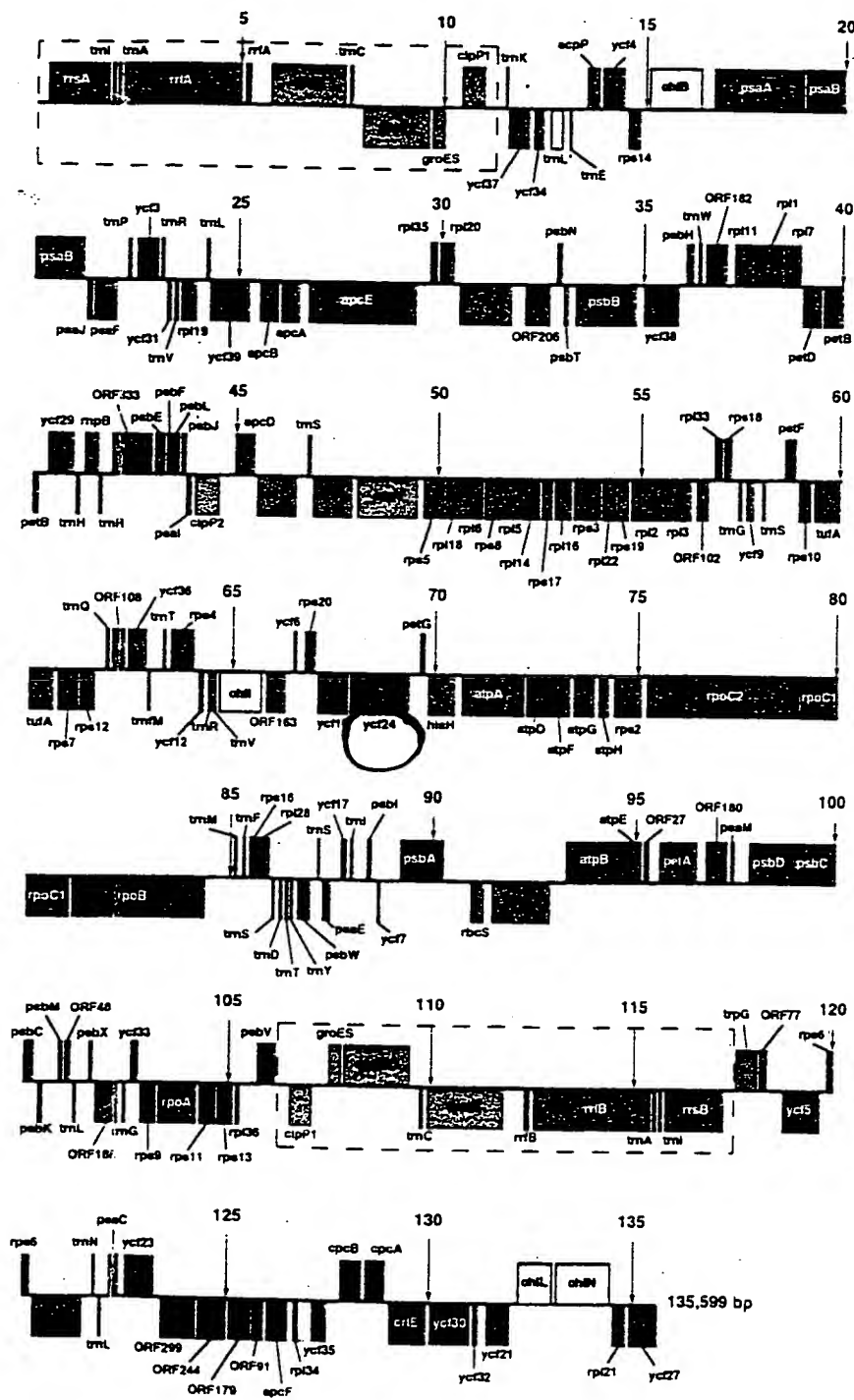
While a full discussion of the sequence characteristics will be presented elsewhere, we should note the special features of cyanelle DNA:

- a single type-I intron located in *trnL*(UUA) in a position that is conserved in many cyanobacteria and all plastid DNAs (Kuhse et al., 1990; Xu et al., 1990);
- a full complement of tRNA genes (36 in total) and the gene *rnpB* (= RNA subunit of RNaseP);
- one set of rRNA genes (16 S, 23 S, and 5 S, with *trnA* and *trnI* genes in the 16 S-23 S spacers) in each IR;
- 37 ribosomal protein genes (18 small-subunit proteins; 19 large-subunit proteins);
- four genes encoding RNA polymerase subunit proteins, and *tufA* encoding translation factor Tu;
- seven genes encoding phycobiliproteins (including all chromophorylated subunits of the phycobilisome);

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Fig. 1: Physical map and gene map of the cyanelle genome of *Cyanophora paradoxa* (opposite page). See table of gene symbols on p. 324.

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- 25 genes encoding subunit proteins of the photosystem I complex (8 proteins) and photosystem II complex (17 subunits);
- seven genes encoding ATPase subunit proteins;
- six genes encoding subunits of the cytochrome *b<sub>f</sub>* complex;
- the *petF* gene encoding type I [2Fe-2S] ferredoxin;
- genes for the molecular chaperones GroEL, GroES, and DnaK located within IR segments; in addition gene *secY* is present, encoding a subunit of the preprotein translocation machinery;
- two different genes (*clpP1* and *clpP2*) encoding ClpP subunits of the Clp protease;
- one gene with homology to *ftsW*, possibly involved in cyanelle division or cell wall biosynthesis;
- 13 identified genes encoding proteins for functions in metabolism;
- cyanobacterial  $L_4S_4$  RuBisCO encoded by an *rbcL-rbcS* operon;
- three ORFs with possible functions in the transcriptional regulation of gene expression (two OmpR family members; one LysR family member);
- 15 ORF and 21 *ycf* (ranging from 27 to 333 codons);
- five ORF/*ycf* with possible functions as membrane transporters;
- an ORF (*ycf17*) encoding a protein of 49 amino acids with strong homology to all members of the CAB/ELIP/HLIP protein superfamily;
- no *ndh* genes or pseudo-*ndh* reading frames (genes for NADH dehydrogenase).

The sequences of four algal plastid DNAs are or will soon be available (Table I): those of the cyanelle genome of *Cyanophora paradoxa* described here, the 191,028-bp plastid DNA from the red alga *Porphyra purpurea* (Reith, 1995; Reith & Murnholland, 1995), the 119,704-bp plastid genome of the diatom *Odontella sinensis* (class *Bacillariophyceae*) (Kowallik et al., 1995), and the 143,170-bp plastid genome of *Euglena gracilis* (Hallick et al., 1993). These sequences complement those of chloroplast DNAs from six higher plant species: the liverwort *Marchantia polymorpha* (Ohyama et al., 1986); the gymnosperm *Pinus thunbergii* (Wakasugi et al., 1994); the monocots *Oryza sativa* (Hiratsuka et al., 1989) and *Zea mays* (140,386 bp; Maier et al., 1995); and the dicots *Nicotiana tabacum* (Shinozaki et al., 1986) and *Epifagus virginiana*, a parasitic, nonphotosynthetic species (Wolfe et al., 1992).

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Table I. Fully sequenced plastid genomes.

Plant Species	Genome Size (bp)	Reference
<b>Algae</b>		
<i>Cyanophora paradoxa</i>	135,599	this article
<i>Porphyra purpurea</i>	119,704	Reith, 1995; Reith & Munholland, 1995
<i>Odontella sinensis</i>	119,680	Kowallik et al., 1995
<i>Euglena gracilis</i>	143,170	Hallick et al., 1993
<i>Chlorella ellipsoidea</i>	155,000	M. Sugiura, personal communication
<b>Higher Plants</b>		
<i>Marchantia polymorpha</i>	121,024	Ohyama et al., 1986
<i>Pinus thunbergii</i>	119,707	Wakasugi et al., 1994
<i>Nicotiana tabacum</i>	155,844	Shinozaki et al., 1986
<i>Epifagus virginiana</i>	70,028	Wolfe et al., 1992
<i>Oryza sativa</i>	134,525	Hiratsuka et al., 1989
<i>Zea mays</i>	140,386	Maier et al., 1995

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ORIGINAL PAPER

## Evidence for a Single Origin of the 35 kb Plastid DNA in Apicomplexans

Paul Denny<sup>a</sup>, Peter Preiser<sup>b</sup>, Don Williamson<sup>b</sup>, and Iain Wilson<sup>b, 1</sup>

<sup>a</sup>Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ

<sup>b</sup>National Institute for Medical Research Mill Hill, London NW71AA

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Gene organization on three selected parts of the 35-kb plastid DNA of the malaria parasite *Plasmodium falciparum* was compared with that of two other apicomplexans, namely *Toxoplasma gondii* and *Eimeria tenella*. This comparison included the characteristic inverted ribosomal RNA repeat. A short segment of DNA from *Theileria annulata* also was included in a separate comparison. Criteria such as the presence or absence of particular genes, their map positions and their sequences, were used to assess whether the apicomplexan plastid DNAs originated from a single origin (a unitary hypothesis for the entire phylum), or whether disparate multiple events were more likely. The results provisionally favour a single origin although clearly this comparison of the apicomplexan pDNAs is still fragmentary. Contrary to the tendency towards homogeneity, evidence was found that the coccidian plastids may have evolved a suppressor mechanism for UGA stop codons.

### Introduction

Traditional systematic observations, as well as phylogenetic studies of small subunit rRNA genes generally agree that apicomplexans are monophyletic (Levine 1987; Van de Peer et al. 1996). Distinct clades correspond to plasmodia, piroplasms, sarcocystans, coccidia etc. (Barta et al. 1991; Gajadhar et al. 1991; Escalante and Ayala 1995). In the present study we have tried to determine by comparative sequence analysis and gene organization whether apicomplexan plastid (pl) DNA originates from a single source.

An estimate of evolutionary rates for rDNAs places the divergence of apicomplexans from dinoflagellates ~900 million years (My) ago in the precambrian period, before the evolutionary radiation

of higher plants and animals (Escalante and Ayala 1995). From our sequence of the 35 kb pDNA of *Plasmodium falciparum* (Wilson et al. 1996) we conjectured it to be of algal origin, acquired through secondary endosymbiosis by an ancient free-living progenitor, possibly a dinoflagellate or some related alveolate (Williamson et al. 1994). Following the principle of Occam's razor, we postulated that when the free-living progenitor became parasitic the algal plastid adopted its present form, the physiological specialization accompanying this change of life style resulting in massive reduction of the algal plastid genome to its vestigial, non-photosynthetic form (Wilson et al. 1994). According to this proposal, all extant apicomplexan lineages should carry pDNA with substantially the same gene content and linkages. An alternative scenario is that the ancestral plastid genome degenerated over a prolonged period of parasitism, allowing lineage-specific differ-

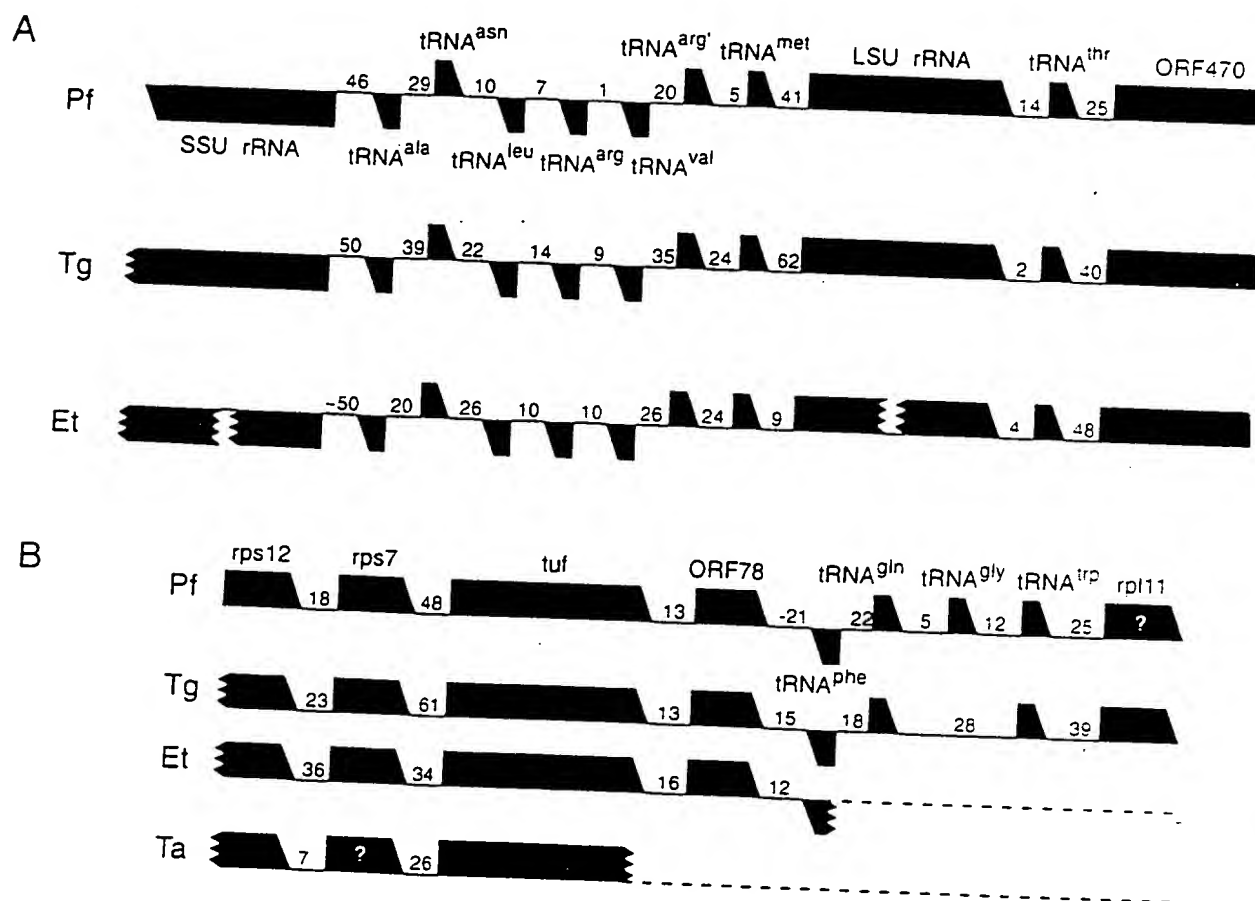
<sup>1</sup> Corresponding author;  
fax 44-181 906 4477;  
e-mail [rwilson@nimr.mrc.ac.uk](mailto:rwilson@nimr.mrc.ac.uk)

ences in gene content or gene organization to emerge with apicomplexan speciation. A third possibility is that each lineage originated independently from separate photosynthetic ancestors. Although this last idea would most likely predict substantial differences in the plastid DNAs of each apicomplexan lineage, this outcome might be countered by convergent evolution, as with mitochondrial DNAs which, although widely different in organization and possibly of multiple origins, tend to have a similar genetic content (Gray and Spencer 1996).

One piece of information already tends to support the first of the three hypotheses outlined above; namely, that the pDNA of *Toxoplasma gondii* resembles that of *Plasmodium*, both in its size and pro-

pensity to form a large distinctive cruciform structure (Borst et al. 1984; Williamson et al. 1985) due to the presence of a rDNA inverted repeat with a novel gene arrangement (Wilson et al. 1993; Kohler et al. 1997). In addition, D. Roos and colleagues (personal communication) have found other genes similar to those on the malarial pDNA (the largely unannotated *T. gondii* sequence has the accession number U87145).

To assess the three hypotheses on the origin of the pDNA further, we have generated our own pDNA sequences from two coccidians, *T. gondii* and *Eimeria tenella*, as well as the piroplasm *Theileria annulata* and compared them with those of *Plasmodium falciparum*.



**Figure 1A.** Diagram (not to scale) showing conservation of gene order on the plastid-like DNAs of *Plasmodium falciparum* (Pf), *Toxoplasma gondii* (Tg), and *Eimeria tenella* (Et). In Pf this region of the pDNA spans approximately 6.3 kb. It includes one copy of the rDNA region of the inverted repeat and genes immediately downstream of the large subunit rRNA (LSU rRNA) – see Wilson et al. (1996). The lengths of intergenic regions are given as the number of nucleotides. Arginyl tRNA genes with different anticodons are distinguished.

**Figure 1B.** Diagram (not to scale) showing the gene arrangement on either side of the *tuf* gene on the pDNAs of *P. falciparum* (Wilson et al. 1996), *T. gondii*, *E. tenella* and *Th. annulata* (Ta). In Pf this region spans ~3.0 kb of the pDNA. As indicated, the tRNA<sup>arg</sup> gene is on the opposite strand from *tuf* in all cases examined. The primers used for PCR did not extend to the dashed regions.

## Results

### Gene Organization

Three regions of the *Plasmodium* 35 kb pDNA with characteristic gene arrangements were selected, and PCR products covering these regions were amplified from three other apicomplexan DNAs. The amplicons were cloned, sequenced and compared with *Plasmodium* pDNA for genetic content, gene organization, and in some cases for nucleotide sequence similarity.

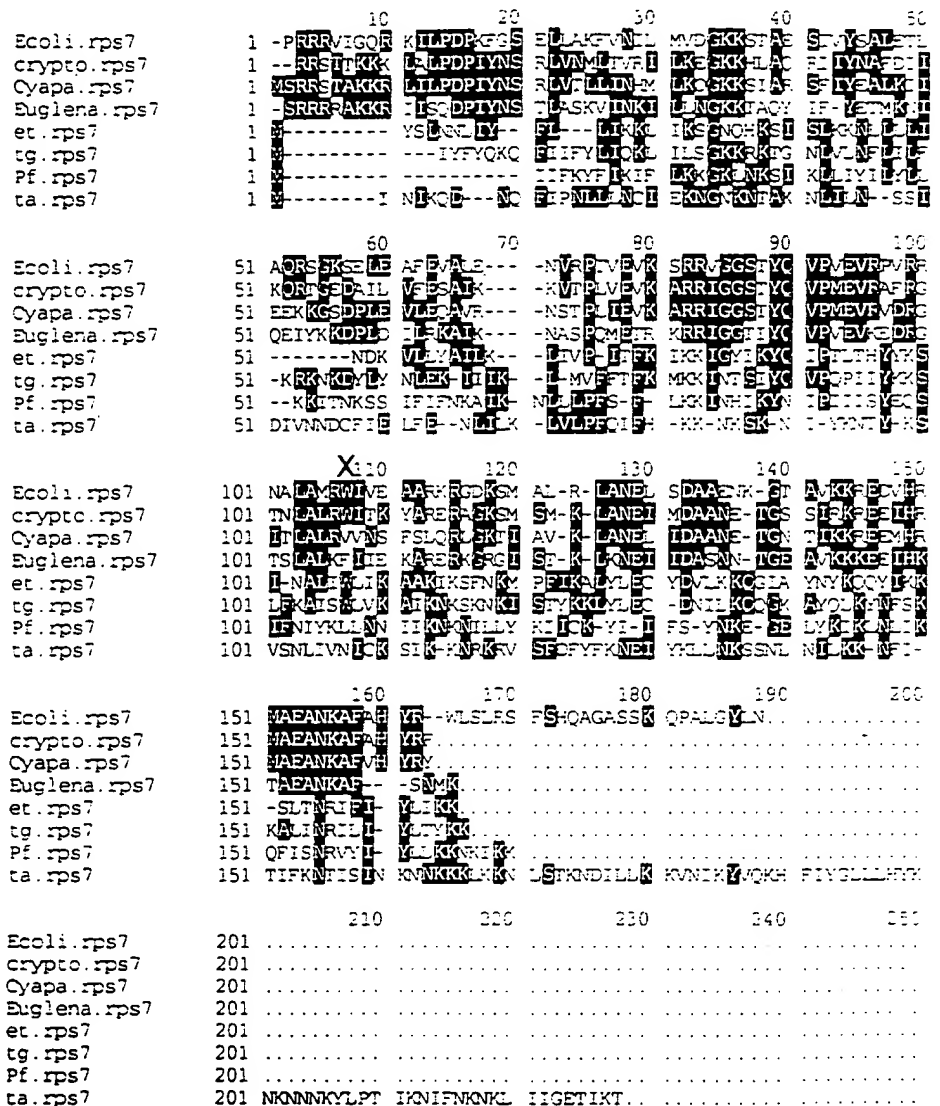
### The Inverted Repeat

The inverted repeat (IR) in *Plasmodium* comprises a novel arrangement of duplicated small and large rRNA genes interspersed with nine tRNA genes, oc-

cupying both strands on this sector of the pDNA (Gardner et al. 1994). Earlier "snap-back" back experiments with DNA from *Toxoplasma* and *Eimeria* were consistent with the presence of an IR and sequence data obtained in the present study confirmed the finding of Roos and colleagues (Accession No. U87145) that the same gene order is conserved (Fig. 1A). It may be noted in passing that a small subunit rRNA gene with similar sequence has also been reported from *Babesia bovis* (Gozar and Bagnara, 1995).

### The ORF470 Region

ORF470 corresponds to the highly conserved gene *yca* 24 that has a limited distribution in known plastids. Our findings, summarized in Fig. 1A, show that in both *Toxoplasma* and *Eimeria* a tRNA<sup>thr(ugu)</sup> gene



and a homologue of the *Plasmodium* ORF470 gene lie downstream of an LSU rRNA gene, just as in *Plasmodium* (Williamson et al. 1994).

### The *tuf* Region

In *Plasmodium*, the ribosomal protein genes *rps* 7 and *rps* 12 precede the *tuf* gene, all three lying on the opposite strand from the adjacent, downstream gene for tRNA<sup>phe</sup>. The same overall arrangement was found on sequenced PCR products derived from the purified pDNA of *Toxoplasma*, and total DNAs from *Eimeria* and *Theileria* (Fig. 1B). Unlike *rps* 12 which is relatively well conserved (~50% amino acid identity with *E. coli*), the putative *rps* 7 gene was poorly conserved in all the apicomplexan DNAs (Fig. 2A). Although ORF78 downstream of the *tuf* gene in *Plasmodium* also was not well conserved in *Toxoplasma* (ORF43) or *Eimeria* (ORF45), conservation of a motif in the latter allowed us tentatively to relate (32% amino acid identity) the apicomplexan sequences to ORF57 found on the pDNA of the euglenoid *Astasia longa* (Gockel et al. 1994) – see Fig. 3A. Another tentative assignment was made for ORF129 located still further downstream of *tuf* in *Plasmodium* (Wilson et al. 1996) – this time a motif in the *Toxoplasma* (ORF123) version led to its provisional identification as the ribosomal protein encoding gene *rpl* 11 [BLAST score = 6.1 e-09 with the gene from *Cyanophora*] (Fig. 3B).

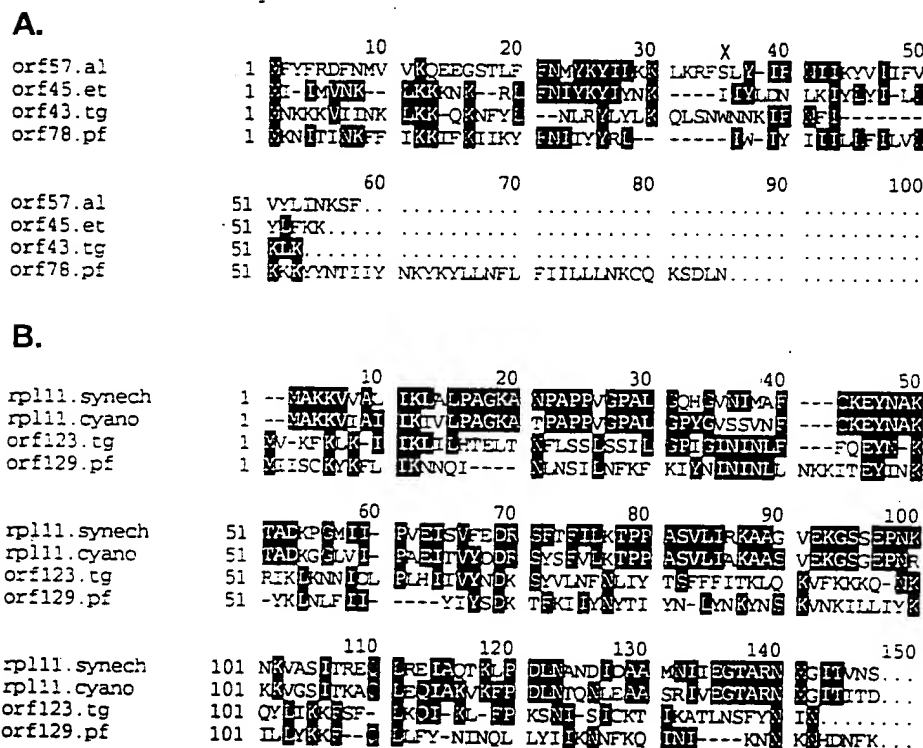
One minor difference in the pDNAs of *Plasmodium* and *Toxoplasma* was noted in the *tuf* region. Of the three contiguous tRNA genes in *Plasmodium* close to but on the opposite strand from tRNA<sup>phe</sup> (i.e. glutamine (Q), glycine (G) and tryptophan (W)), only those for Q and W were present in *Toxoplasma*. Apart from this exception, the order of all the contiguous genes we provisionally identified was conserved in *Plasmodium*, *Toxoplasma*, *Eimeria* and *Theileria*.

As well as gene order, the sequences of the genes on the apicomplexan pDNAs were more similar to each other than to other plastid versions. For example, the N-terminus of the hypothetical translation product of *ycf* 24 of *Porphyra purpurea* was more similar to the cyanobacterial version (*Synechocystis* sp.) than were the apicomplexan ones (compare amino acid blocks 20–30; 30–40; 50–60; 70–80 in Fig. 4). Yet the overall homology was readily apparent over the rest of the protein.

The conserved nature of the apicomplexan protein sequences, as well as their unique gene order, support the idea that the pDNAs originated from a single source.

### Tryptophan Codons

Unlike *Plasmodium*, the coccidians *Toxoplasma* and *Eimeria* appeared to use an opal stop codon (UGA) at a few sites in conserved tryptophan positions. As shown in Figure 4, this occurred at different single



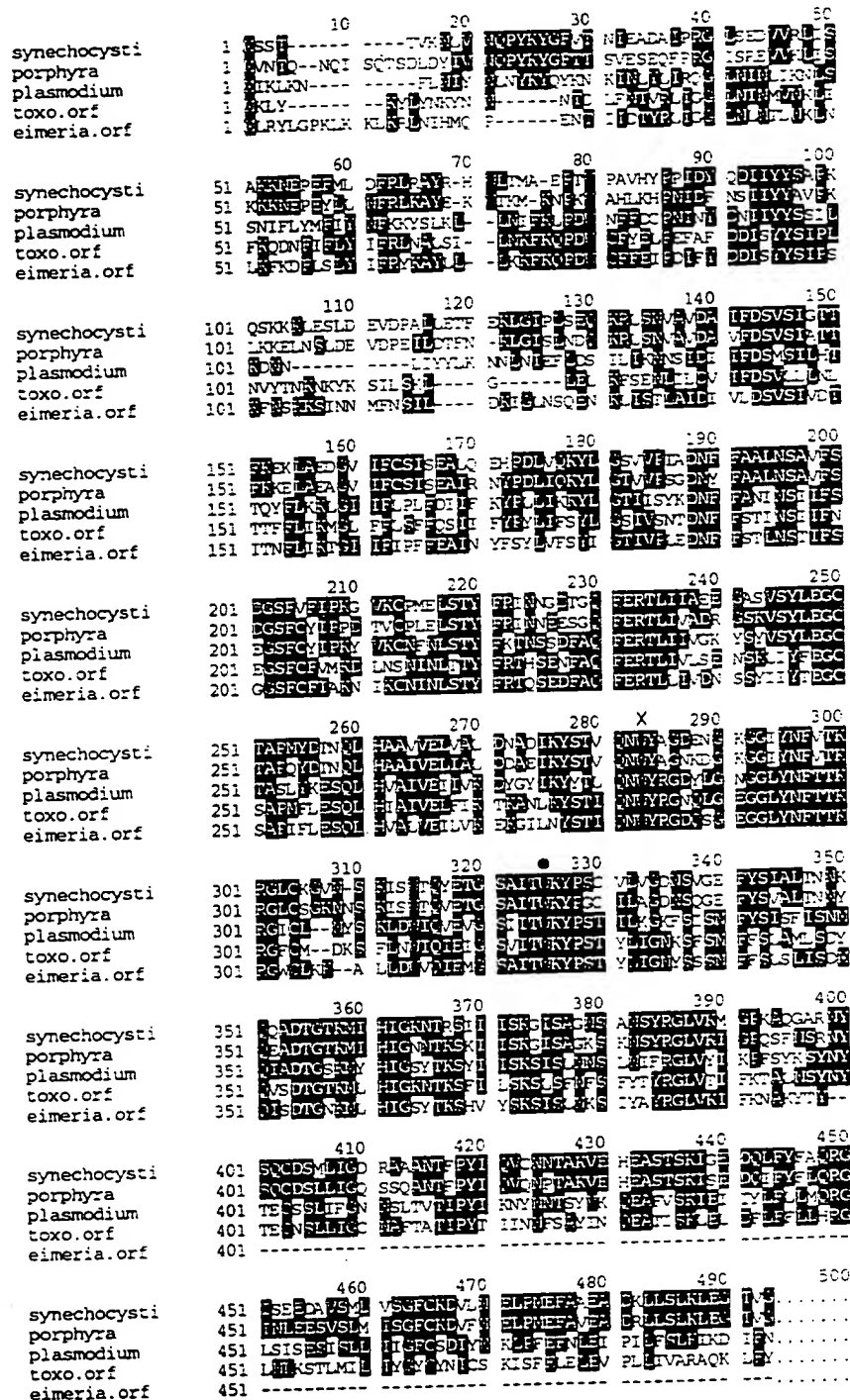
**Figure 3.**

**A)** An alignment showing similarity between ORF57 from *Astasia longa* (Gockel et al. 1994) and the small ORF lying downstream from the *tuf* gene on the apicomplexan pDNAs. A cross indicates the position of a tryptophan in the *T. gondii* sequence encoded by a UGA "stop codon".

**B)** An alignment of the putative apicomplexan *rpl* 11 sequences from *P. falciparum* (ORF129) and *T. gondii* (ORF123) with those of *Synechocystis* spp. (P36237) and *Cyanophora paradoxa* (P48126).

sites (cross and filled circle, respectively) in the *Toxoplasma* and *Eimeria* versions of *ycf 24*. The *Toxoplasma* opal stop codon occurred at the same site in the sequence obtained independently by Roos et al. (Accession No. U87145). In both *Toxoplasma* and *Eimeria*, the sequence around the unusual codon was highly conserved and interpretation of the opal stop as a tryptophan codon restored conserved C-

terminal sequences. In the case of *rps 7* (a less well conserved protein) *Toxoplasma* again used a UGA codon in a tryptophan position that is often conserved outside the apicomplexans (Fig. 2). We found no evidence for RNA editing of any of these positions (data not shown) and infer that the coccidian plastid has evolved a mechanism for UGA stop codon suppression (see Discussion).



**Figure 4.** An alignment of the hypothetical translation products of apicomplexan versions of Pf ORF470 (*ycf 24*) with those of the plastid from *Porphyra purpurea* (U38804) and the cyanobacterium *Synechocystis* sp. (D64004). Our sequences for *T. gondii* and *E. tenella* were incomplete downstream of a conserved region used to design the PCR primers at the C-terminus. For the purposes of this figure, the region downstream of position 398 was filled-in for *T. gondii* using the database entry (U87145) of Roos and colleagues. Tryptophans predicted from UGA "stop codons" are indicated by a cross for *T. gondii* and filled circle for *E. tenella*.



## Intergenic Regions

Intergenic regions of the apicomplexan pDNA were extremely A-T-rich and more heterogeneous than the open reading frames, indels of different length being found. But because intergenic regions (other than control sequences) are less likely to be constrained by natural selection than functional open reading frames, these findings are probably not relevant to the hypothesis being examined, namely whether all the pDNAs evolved from a single source.

## Discussion

We have shown that portions of the pDNA of four apicomplexan genera (covering about one third of the total length of *Plasmodium* pDNA), have a highly conserved gene content, gene order and gene sequences. This favours the hypothesis that they evolved from a single photosynthetic progenitor. The 35 kb pDNA is about one fifth the size of a conventional plastid DNA molecule and to have reached its present form and novel gene arrangement it must have undergone numerous deletions and rearrangements. As the apicomplexans are believed to have diverged as long ago as 800 My (Escalante and Ayala 1995), it seems unlikely their pDNAs became reduced to the present novel arrangement through convergent evolution from different origins. It may be noted in this regard that the vestigial pDNAs of two parasitic higher plants, *Epifagus virginiana* (beechdrops) (Wolfe et al. 1992a) and *Conopholis americana* (squawroot) (Wimpee et al. 1992) from the family Orobanchaceae (of the parasitic branch of the Scrophulariales), now differ considerably in gene content and organization as the result of extensive deletions and rearrangements incurred since they diverged from their congeners over the much shorter period of some 5–50 My (Muller, 1981; Wolfe et al. 1992b).

Like ourselves, D. Roos and his colleagues have found UGA "stop codons" at a few conserved tryptophan residues in the pDNA of *Toxoplasma* (personal communication). Thus it would be doubly interesting to confirm translation of the gene products of *ycf 24* and *rps 7* and to clarify the proposed suppressor mechanism for these UGA stop codons. Unfortunately, at present the reagents required to demonstrate protein expression (for example, specific antibodies) are unavailable. A number of different suppressor mechanisms for stop codons have been recorded in other systems; these include mutations in the peptidyl-transferase region of the large subunit rRNA (Jemiolo et al. 1995), and specific

deletions in the small subunit rRNA (Murgola et al. 1988). Neither of these seems applicable in the present case (Denny, unpublished data). Likewise, various mutations in tryptophan tRNAs can act as suppressors of UGA stop codons (Hirsh 1971; Hirsh and Gold 1971). This list can be extended to release factors and ribosomal proteins, but for the present we have no clear indication of how suppression might be achieved in the coccidial plastid organelle and further work will be required to clarify this issue.

A recent phylogenetic analysis of the apicomplexan plastid *tuf* gene (Kohler et al. 1997) placed it with green plastids rather than with those of the red algae we originally suggested might be closest based on the then known distribution of ORF470 (*ycf 24*) (Williamson et al. 1994). But bootstrap support among the plastid clades is weak in such analyses and so there is as yet no clear support for any one plastid group furnishing the potential endosymbiont whose pDNA has been maintained in a vestigial form in apicomplexans.

The highly conserved nature of the gene content of the vestigial pDNA in four genera of apicomplexans, despite their evolution of different life styles over a long evolutionary time span, suggests the organelle has an essential function (McFadden and Waller 1997; Kohler et al. 1997). Direct evidence for apicomplexan plastid proteins remains to be gathered, but transcriptional studies have confirmed that the *rRNA*, *tRNA*, *tuf*, *ycf 24*, and *rps 7* genes mentioned here are all transcribed in erythrocytic forms of *Plasmodium* and tachyzoites of *Toxoplasma* (Wilson et al. 1996, and data not shown).

To strengthen our postulate that the pDNA of apicomplexans comes from a single source, information should be gathered from other members of the phylum, especially those that could be considered less specialized, such as the gregarines which infest invertebrate lineages. For the present, however, we conclude that apicomplexan parasites of vertebrates, despite their extensive evolutionary divergence, originated from a single photosynthetic ancestral cell whose pDNA underwent massive deletion and rearrangement, either leading to or following the parasitic event.

## Methods

**DNA.** All the DNA sequences reported in this paper were obtained from the following starting materials. Total DNA from *P. falciparum* – clone C10 (Hempelmann et al. 1981) was extracted and fractionated on caesium chloride/4', 6-diamidino-2-phenylindole (DAPI) gradients to yield isolated 35 kb DNA, as pre-

Table 1. Oligonucleotide primers for amplification of apicomplexan plastid DNAs.

Segment of circle	PCR primers pairs
<i>a. Toxoplasma gondii</i>	
rRNA/tRNA Inverted Repeat-ORF470	5' TACGGCTACCTTGTTACGACTTC and 5' GCGGTAATACAGAAAATGCAAG <sup>1</sup>
	5' AAGAACATACCAATCCACC and 5' CTATTTACCCGAATATTT
	5' GCGAAATTCCTTGTCGGGTAAGTTCC <sup>2</sup> and 5' TTTT/CT/CGG/ATCCTCTCGTAC <sup>2</sup>
	5' GTTCGCCTATTAAAGCGATACGTGAGCTGGG and 5' ATCACATTGAG/CA/TATAATT
	5' GGAGGTAGAGTAAAAGATTTACCAGG and 5' GGTAGAGCAATGGATTGAAG
	5' CTTCAATCCATTGCTCTACC and 5' GTGAGTATAGTTTAG
rps 12-rps 7-tuf-tRNAs-rpl 11	
<i>b. Eimeria tenella</i>	
rRNA/tRNA Inverted Repeat-ORF470	5' CTGAGCCAGGATCAAACCTC and 5' GGAAAAGTGCTTCTAAG
	5' CTTAGAAGCAGTTTTCC and 5' TTTCTCACTTATATGTTGTC
	5' GTTCGCCTATTAAAGCGATACGTGAGCTGGG and 5' ATCACATTGAG/CA/TATAATT
rps 12-rps 7-tuf-tRNA <sup>pro</sup>	5' GGAGGTAGAGTAAAAGATTTACCAGG and 5' GGTAGAGCAATGGATTGAAG
<i>c. Theileria annulata</i>	
rps 12-URF-tuf	5' GGAGGTAGAGTAAAAGATTTACCAGG and 5' ATCTTCTTTATTTAAAAA/TAC

<sup>1</sup> Gozar and Bagnara (1995).<sup>2</sup> Beckers et al. (1995).

viously described (Gardner et al. 1988). *T. gondii* (*Toxoplasma* - RH strain) was obtained either from infected cotton rats (courtesy of Dr. Judith Smith, Leeds University) or from infected cultures of the dog epithelial cell line MDCK. Following buoyant density centrifugation of total *Toxoplasma* DNA as described above, the 35 kb DNA homologue was identified as a low density satellite band. This was removed by side puncture of the centrifuge tube, purified as a pellet by further centrifugation, and shown to cross-hybridize in Southern blots with probes from the *Plasmodium* 35 kb DNA. Total DNA was also extracted from sporozoites of *Eimeria tenella* - the H strain was supplied by Dr Fiona Tomley, Institute of Animal Health, Compton, and from trophozoites of *Theileria annulata* in bovine blood - the Hissar strain was supplied by Dr Roger Hall, Dept. Biology, York University.

**Polymerase chain reaction (PCR).** Oligonucleotide primers were prepared based on conserved regions of the *Plasmodium* 35 kb pDNA or from other published sequences (Table 1). PCR products were generated using either the *Toxoplasma* circular DNA or, in the cases of *Eimeria* and *Theileria* total genomic DNAs as template. PCR reactions were carried out with 20 ng *Toxoplasma* pDNA, 150 ng total *Eimeria* DNA, or 300 ng total *Theileria* DNA as templates. Approximately 50 pmol of each primer was added to reaction mixtures containing 2.5 mM MgCl<sub>2</sub>, x1 PCR buffer (Promega) and 1 µl (5 units) Amplitaq (Cetus) in 100 µl total volume. Amplification was carried out for 35 cycles as follows: 95 °C for 30 sec, 40 °C for 60 sec, 72 °C for 120 sec. As negative controls, total DNA was used from *Leishmania guyanensis* B8 (courtesy Dr Douglas Barker, Molteno Laboratories, Cambridge), or from MDCK cells.

**Southern Hybridization.** DNAs were digested at 37 °C overnight with *Hind*III (Gibco/BRL), and the restriction fragments from approximately 1 µl of total DNA from *P. falciparum*, *T. gondii*, *E. tenella* and *Th. annulata*, as well as 20 ng of isolated *P. falciparum* pDNA, were separated by electrophoresis in 0.8% agarose in the presence of 0.6 µg ml<sup>-1</sup> Ethidium Bromide. The DNA was transferred under alkaline conditions to Hybond N+ membranes, according to the manufacturer's instructions and hybridization was carried out using <sup>32</sup>P-labelled probes prepared by the random primer method (as described in the Prime-It II Kit, Stratagene). In some experiments a lower stringency was used for hybridization and washing (45–50 °C with high salt).

**Sequence analysis.** PCR products were purified using the Wizard PCR Preps kit (Promega) and sequenced on one strand by the Sanger di-deoxy chain termination method (Sambrook et al. 1989)

using the Sequenase Version 2.0 kit (USB). If necessary, terminal dideoxy-transferase (TdT) was included, as described previously (Fawcett and Bartlett, 1990). The sequences were assembled using the Staden-Plus programme SAP (Amersham). Reverse strands were sequenced by fluorescent dye-terminated thermo-cycle sequencing (Perkin Elmer) using an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). Sequences were further analysed using the Sequence Analysis Software Package (Genetics Computer Group, Wisconsin version 7.0) (Devereux et al. 1984). EMBL database accession numbers for data presented here are as follows: *Plasmodium* X95275, X95276; *Toxoplasma* Y11430, Y11431; *Eimeria* Y12332, Y12333; *Theileria* Y11429.

## Acknowledgements

We are grateful to Dr Judith Smith, Leeds University; Dr Mary Pudney, Glaxo/Wellcome; Dr Fiona Tomley, Institute of Animal Health, Compton and Dr R. Hall, Dept. Biology, York University, U.K. for providing *Toxoplasma* isolates and *Eimeria* and *Theileria* materials. We also thank Irene Ling and Malcolm Strath (N.I.M.R.) for carrying out the automated sequencing work. P.D. was the recipient of an British MRC Studentship award. This work was also supported by the UNDP/World Bank/WHO Special Programme for Research in Tropical Diseases (TDR).

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**APPENDIX D**

**Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI  
database**

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

<http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?CMD=search&DB=nucleotide>  
search : ycf24

- 1: D64004  
Synechocystis sp. PCC 6803 DNA, complete genome, section:23/27, 2868767-3002965  
gi|1001701|dbj|D64004.1|SYCSLRF[1001701]
- 2: NC\_004113  
Thermosynechococcus elongatus BP-1, complete genome  
gi|22297544|ref|NC\_004113.1|[22297544]
- 3: NC\_003272  
Nostoc sp. PCC 7120 complete genome  
gi|17227497|ref|NC\_003272.1|[17227497]
- 4: NC\_000911  
Synechocystis sp. PCC 6803, complete genome  
gi|16329170|ref|NC\_000911.1|[16329170]
- 5: NC\_000916  
Methanobacterium thermoautotrophicum str. Delta H complete genome  
gi|15678031|ref|NC\_000916.1|[15678031]
- 6: NC\_001799  
Toxoplasma gondii apicoplast, complete genome  
gi|11496534|ref|NC\_001799.1|[11496534]
- 7: NC\_001713  
Odontella sinensis chloroplast, complete genome  
gi|11467432|ref|NC\_001713.1|[11467432]
- 8: NC\_001675  
Cyanophora paradoxa cyanelle, complete genome  
gi|11467282|ref|NC\_001675.1|[11467282]
- 9: NC\_000925  
Porphyra purpurea chloroplast, complete genome  
gi|11465652|ref|NC\_000925.1|[11465652]
- 10: NC\_000926  
Guillardia theta chloroplast, complete genome  
gi|11467607|ref|NC\_000926.1|[11467607]
- 11: NC\_001840  
Cyanidium caldarium chloroplast, complete genome  
gi|11465393|ref|NC\_001840.1|[11465393]
- 12: AP005370  
Thermosynechococcus elongatus BP-1 DNA, complete genome, section 2/9  
gi|22294033|dbj|AP005370.1|[22294033]
- 13: AE000884  
Methanobacterium thermoautotrophicum from bases 1050856 to 1062059 (section 90 of 148)  
of the complete genome  
gi|2622242|gb|AE000884.1|[2622242]
- 14: AP003589  
Nostoc sp. PCC 7120 DNA, complete genome, section 9/19  
gi|17131372|dbj|AP003589.1|[17131372]

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

15: BI437350  
gc59d11.y1 Moss EST library PPN Physcomitrella patens cDNA clone  
PEP\_SOURCE\_ID:PPN200721 5' similar to SW:YC24\_ODOSI P49530 HYPOTHETICAL  
54.3 KD PROTEIN YCF24 ;, MRNA sequence  
gi|15262040|gb|BI437350.1|[15262040]

16: Z67753  
Odontella sinensis complete chloroplast genome  
gi|1185127|emb|Z67753.1|OSCHLPLXX[1185127]

17: AJ132267  
Skeletonema costatum chromoplast ycf24 gene, partial  
gi|4210403|emb|AJ132267.1|SCO132267[4210403]

18: AF022186  
Cyanidium caldarium strain RK1 chloroplast, complete genome  
gi|6466296|gb|AF022186.2|AF022186[6466296]

19: AF138960  
Neospora caninum ycf24 protein (ycf24) gene, partial cds; DNA dependent RNA polymerase beta subunit (rpoB) gene, complete cds; and DNA dependent RNA polymerase beta subunit' (rpoC1) gene, partial cds, plastid genes for plastid products  
gi|6492292|gb|AF138960.1|AF138960[6492292]

20: U87145  
Toxoplasma gondii chloroplast, complete genome  
gi|5231237|gb|U87145.2|U87145[5231237]

21: AF095904  
Toxoplasma gondii ycf24 protein (ycf24) gene, partial cds; DNA dependent RNA polymerase beta subunit (rpoB) gene, complete cds; and DNA dependent RNA polymerase beta' subunit (rpoC1) gene, plastid genes encoding plastid proteins, partial cds  
gi|4336507|gb|AF095904.1|AF095904[4336507]

22: AF041468  
Guillardia theta complete plastid genome  
gi|3602932|gb|AF041468.1|[3602932]

23: U38804  
Porphyra purpurea chloroplast, complete genome  
gi|1276652|gb|U38804.1|PPU38804[1276652]

24: D90812  
E.coli genomic DNA, Kohara clone #321(38.1-38.4 min.)  
gi|1742768|dbj|D90812.1|[1742768]

25: D90811  
E.coli genomic DNA, Kohara clone #320(37.9-38.3 min.)  
gi|1742754|dbj|D90811.1|[1742754]

26: U30821  
Cyanophora paradoxa cyanelle, complete genome  
gi|1016083|gb|U30821.1|CPU30821[1016083]

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

1: D64004. Synechocystis sp....[gi:1001701]

LOCUS SYCSLRF 134199 bp DNA linear BCT 08-FEB-2003  
DEFINITION Synechocystis sp. PCC 6803 DNA, complete genome, section:23/27,  
2868767-3002965.  
ACCESSION D64004 AB001339 BA000022  
VERSION D64004.1 GI:1001701  
KEYWORDS .  
SOURCE Synechocystis sp. PCC 6803  
ORGANISM Synechocystis sp. PCC 6803  
Bacteria; Cyanobacteria; Chroococcales; Synechocystis.  
REFERENCE 1  
AUTHORS Kaneko,T., Tanaka,A., Sato,S., Kotani,H., Sazuka,T., Miyajima,N.,  
Sugiura,M. and Tabata,S.  
TITLE Sequence analysis of the genome of the unicellular cyanobacterium  
Synechocystis sp. strain PCC6803. I. Sequence features in the 1 Mb  
region from map positions 64% to 92% of the genome  
JOURNAL DNA Res. 2 (4), 153-166 (1995)  
MEDLINE 96127529  
PUBMED 8590279  
REFERENCE 2  
AUTHORS Kaneko,T., Sato,S., Kotani,H., Tanaka,A., Asamizu,E., Nakamura,Y.,  
Miyajima,N., Hirose,M., Sugiura,M., Sasamoto,S., Kimura,T.,  
Hosouchi,T., Matsuno,A., Muraki,A., Nakazaki,N., Naruo,K.,  
Okumura,S., Shimpo,S., Takeuchi,C., Wada,T., Watanabe,A.,  
Yamada,M., Yasuda,M. and Tabata,S.  
TITLE Sequence analysis of the genome of the unicellular cyanobacterium  
Synechocystis sp. strain PCC6803. II. Sequence determination of the  
entire genome and assignment of potential protein-coding regions  
JOURNAL DNA Res. 3 (3), 109-136 (1996)  
MEDLINE 97061201  
PUBMED 8905231  
REFERENCE 3 (bases 1 to 134199)  
AUTHORS Tabata,S.  
TITLE Direct Submission  
JOURNAL Submitted (30-AUG-1995) Satoshi Tabata, Kazusa DNA Research  
Institute, The First Laboratory for Plant Gene Research; Yana  
1532-3, Kisarazu, Chiba 292-0812, Japan  
(E-mail:tabata@kazusa.or.jp, URL:http://www.kazusa.or.jp/cyano/,  
Tel:81-438-52-3933(ex.2330); Fax:81-438-52-3934)  
COMMENT Potential protein coding regions were assigned on the basis of  
similarity search of the ORFs and GeneMark analysis.  
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/gene="ycf24"  
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## APPENDIX D

### Excerpts from 26 *yc24* gene product and gene sequences retrieved from NCBI database

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ALLETFEKLGIP LSEQKRLSNVAVDAIFDSV SIGTT FKEKLAEDGVIFCSI SEALQEH  
PDLVQKYLGSVVPTADNFFAALNSAVFSDGSFVFIPKGVKCPMELSTYFRINNGDTGQ  
FERTLIIAEEGASVSYLEGCTAPMYDTNQLHAAVVELVALDNADIKYSTVQNWYAGDE  
NGKGGIYNFVTKRGLCKGVNSKISWTQVETGSAITWKYPSCVLVGDNSVGEFY SIALT  
NNKQQADTGTKMIHIGKNTRSIIISKGISAGNSANSYRGLVKMGPKAQGARNYSQCDS  
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FCKDVLNELPMEFAAEADKLLSLKLEGTVG"
```

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

2: NC\_004113. Thermosynechococc... [gi:22297544]

Links

LOCUS NC\_004113 2593857 bp DNA circular BCT 10-DEC-2002  
DEFINITION Thermosynechococcus elongatus BP-1, complete genome.  
ACCESSION NC\_004113  
VERSION NC\_004113.1 GI:22297544  
KEYWORDS .  
SOURCE Thermosynechococcus elongatus BP-1  
ORGANISM Thermosynechococcus elongatus BP-1  
Bacteria; Cyanobacteria; Chroococcales; Thermosynechococcus.  
REFERENCE 1 (bases 1 to 2593857)  
AUTHORS Nakamura,Y., Kaneko,T., Sato,S., Ikeuchi,M., Katoh,H., Sasamoto,S.,  
Watanabe,A., Iriguchi,M., Kawashima,K., Kimura,T., Kishida,Y.,  
Kiyokawa,C., Kohara,M., Matsumoto,M., Matsuno,A., Nakazaki,N.,  
Shimpo,S., Sugimoto,M., Takeuchi,C., Yamada,M. and Tabata,S.  
TITLE Complete genome structure of the thermophilic cyanobacterium  
Thermosynechococcus elongatus BP-1  
JOURNAL DNA Res. (2002) In press  
REFERENCE 2 (bases 1 to 2593857)  
AUTHORS Kaneko,T.  
TITLE Direct Submission  
JOURNAL Submitted (05-JUN-2002) Takakazu Kaneko, Kazusa DNA Research  
Institute, The First Laboratory for Plant Gene Research; 2-6-7  
Kazusa-kamatari, Kisarazu, Chiba 292-0812, Japan  
(E-mail:kaneko@kazusa.or.jp,  
URL:http://www.kazusa.or.jp/cyano/Thermo/  
Tel:81-438-52-3935(ex.2338), Fax:81-438-52-3934)  
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final  
NCBI review. The reference sequence was derived from BA000039.  
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/db\_xref="taxon:197221"  
...  
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CDS complement(486379..487815)  
/gene="ycf24"  
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RDVFNQLPMEFAVEADRLLSLKLEGSVG"

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

3: NC\_003272. Nostoc sp. PCC 71...[gi:17227497]

### Links

LOCUS NC\_003272 6413771 bp DNA circular BCT 10-DEC-2002  
 DEFINITION Nostoc sp. PCC 7120 complete genome.  
 ACCESSION NC\_003272  
 VERSION NC\_003272.1 GI:17227497  
 KEYWORDS .  
 SOURCE Nostoc sp. PCC 7120  
 ORGANISM Nostoc sp. PCC 7120  
 Bacteria; Cyanobacteria; Nostocales; Nostocaceae; Nostoc.  
 REFERENCE 1  
 AUTHORS Kaneko,T., Nakamura,Y., Wolk,C.P., Kuritz,T., Sasamoto,S.,  
 Watanabe,A., Iriguchi,M., Ishikawa,A., Kawashima,K., Kimura,T.,  
 Kishida,Y., Kohara,M., Matsumoto,M., Matsuno,A., Muraki,A.,  
 Nakazaki,N., Shimpo,S., Sugimoto,M., Takazawa,M., Yamada,M.,  
 Yasuda,M. and Tabata,S.  
 TITLE Complete genomic sequence of the filamentous nitrogen-fixing  
 cyanobacterium Anabaena sp. strain PCC 7120  
 JOURNAL DNA Res. 8 (5), 205-213 (2001)  
 MEDLINE 21595285  
 PUBMED 11759840  
 REFERENCE 2 (bases 1 to 6413771)  
 AUTHORS Kaneko,T.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-MAY-2001) Takakazu Kaneko, Kazusa DNA Research  
 Institute, The First Laboratory for Plant Gene Research; Yana  
 1532-3, Kisarazu, Chiba 292-0812, Japan  
 (E-mail:kaneko@kazusa.or.jp,  
 URL:http://www.kazusa.or.jp/cyanobase/,  
 Tel:81-438-52-3935(ex.2338), Fax:81-438-52-3934)  
 COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final  
 NCBI review. The reference sequence was derived from BA000019.  
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 CDS 2991689..2993128  
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 ELIKKYLGSVPIADNYFAALNAAVFS DGSFVYIPKGVKCPMELSTYFRINS GDTGQF  
 ERTLIVAEEGSYVSYLEGCTAPMYDS NQLHAAVVELVALDNAEIKYSTVQNWYAGDAN  
 GKGGIYNFVTKRGLCQGVNSKISWTQVETGSAITWKYPSCVLVGDNSVGEFY SVALTN  
 NMQQADTGTKMIHIGKNTRSTIISKGISAGQSSNSYRGLVKINPTAKGARNYSQCDSM  
 LIGDNAHANTFPYIQVQNN TGKVEHEASTKIGEDQLFFFAQRGISSEDAISMMSGF

**APPENDIX D**

**Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database**

CKDVFNQLPMEFAVEADKLLSLKLEGSVG"

# APPENDIX D

## Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

4: NC\_000911. *Synechocystis* sp....[gi:16329170]

### Links

LOCUS NC\_000911 3573470 bp DNA circular BCT 09-DEC-2002  
 DEFINITION *Synechocystis* sp. PCC 6803, complete genome.  
 ACCESSION NC\_000911  
 VERSION NC\_000911.1 GI:16329170  
 KEYWORDS .  
 SOURCE *Synechocystis* sp. PCC 6803  
 ORGANISM *Synechocystis* sp. PCC 6803  
 Bacteria; Cyanobacteria; Chroococcales; *Synechocystis*.  
 REFERENCE 1 (bases 1 to 3573470)  
 AUTHORS Kaneko,T., Tanaka,A., Sato,S., Kotani,H., Sazuka,T., Miyajima,N., Sugiura,M. and Tabata,S.  
 TITLE Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. I. Sequence features in the 1 Mb region from map positions 64% to 92% of the genome  
 JOURNAL DNA Res. 2 (4), 153-166 (1995)  
 MEDLINE 96127529  
 PUBMED 8590279  
 REFERENCE 2 (bases 1 to 3573470)  
 AUTHORS Kaneko,T., Sato,S., Kotani,H., Tanaka,A., Asamizu,E., Nakamura,Y., Miyajima,N., Hirose,M., Sugiura,M., Sasamoto,S., Kimura,T., Hosouchi,T., Matsuno,A., Muraki,A., Nakazaki,N., Naruo,K., Okumura,S., Shimpo,S., Takeuchi,C., Wada,T., Watanabe,A., Yamada,M., Yasuda,M. and Tabata,S.  
 TITLE Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions  
 JOURNAL DNA Res. 3 (3), 109-136 (1996)  
 MEDLINE 97061201  
 PUBMED 8905231  
 REFERENCE 3 (bases 1 to 3573470)  
 AUTHORS Tabata,S.  
 TITLE Direct Submission  
 JOURNAL Submitted (28-JUN-1996) Satoshi Tabata, Kazusa DNA Research Institute, The First Laboratory for Plant Gene Research; Yana 1532-3, Kisarazu, Chiba 292-0812, Japan  
 (E-mail:tabata@kazusa.or.jp, URL:http://www.kazusa.or.jp/cyano/, Tel:81-438-52-3933(ex.2330), Fax:81-438-52-3934)  
 COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from BA000022.  
 COMPLETENESS: full length.  
 FEATURES Location/Qualifiers

...

gene 2871674..2873116  
 /gene="ycf24"  
 /note="locus\_tag: slr0074"  
 CDS 2871674..2873116  
 /gene="ycf24"  
 /codon\_start=1  
 /transl\_table=11  
 /product="ABC transporter subunit"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```
/protein_id="NP_442472.1"  
/db_xref="GI:16331744"  
/translation="MSSTTVKNLVNQPYPYGFVTNIEADAI PRGLSEDVVRLISAKKN  
EPEFMLDFRLRAYRHWLTMAEPTWPAVHYPPIDYQDI IYYSAPKQSKKKLES LDEVDP  
ALLETFEKLGIPLSEQKRLSNVAVDAIFDSV SIGTTFKEKLAEDGVIFCSISEALQEH  
PDLVQKYLGSVVPTADNFFAALNSAVFSDGSFVFIPKGVKCPMELSTYFRINNGDTGQ  
FERTLIIAEEGASVSYLEGCTAPMYDTNQLHAAVVVELVALDNADI KYSTVQNWYAGDE  
NGKGGIYNFVTKRGLCKGVNSKISWTQVETGSAITWKYPSCVLVGDNSVGEFYSIALT  
NNKQQADTGTKMIHIGKNTRSI IISKGISAGNSANSYRGLVKMGPKAQGARNYSQCDS  
MLIGDRAAANTFPYIQVDNNTAKVEHEASTSKIGEDQLFYFAQRGISEEDAVSMLVSG  
FCKDVLNELPMEFAAEADKLLSLKLEGTVG"
```

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

5: NC\_000916. Methanobacterium ...[gi:15678031]

### Links

LOCUS NC\_000916 1751377 bp DNA circular BCT 10-DEC-2002  
 DEFINITION Methanobacterium thermoautotrophicum str. Delta H complete genome.  
 ACCESSION NC\_000916  
 VERSION NC\_000916.1 GI:15678031  
 KEYWORDS .  
 SOURCE Methanothermobacter thermautotrophicus str. Delta H  
 (Methanobacterium thermoautotrophicum str. deltaH)  
 ORGANISM Methanothermobacter thermautotrophicus str. Delta H  
 Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales;  
 Methanobacteriaceae; Methanothermobacter.  
 REFERENCE 1 (bases 1 to 1751377)  
 AUTHORS Smith,D.R., Doucette-Stamm,L.A., Deloughery,C., Lee,H.-M.,  
 Dubois,J., Aldredge,T., Bashirzadeh,R., Blakely,D., Cook,R.,  
 Gilbert,K., Harrison,D., Hoang,L., Keagle,P., Lumm,W., Pothier,B.,  
 Qiu,D., Spadafora,R., Vicare,R., Wang,Y., Wierzbowski,J.,  
 Gibson,R., Jiwani,N., Caruso,A., Bush,D., Safer,H., Patwell,D.,  
 Prabhakar,S., McDougall,S., Shimer,G., Goyal,A., Pietrovski,S.,  
 Church,G.M., Daniels,C.J., Mao,J.-i., Rice,P., Nolling,J. and  
 Reeve,J.N.  
 TITLE Complete genome sequence of Methanobacterium thermoautotrophicum  
 deltaH: functional analysis and comparative genomics  
 JOURNAL J. Bacteriol. 179 (22), 7135-7155 (1997)  
 MEDLINE 98037514  
 PUBMED 9371463  
 REFERENCE 2 (bases 1 to 1751377)  
 AUTHORS NCBI Microbial Genomes Annotation Project.  
 TITLE Direct Submission  
 JOURNAL Submitted (25-JUN-2001) National Center for Biotechnology  
 Information, NIH, Bethesda, MD 20894, USA  
 FEATURES Location/Qualifiers  
 source 1..1751377  
 /organism="Methanothermobacter thermautotrophicus str.  
 Delta H"  
 /strain="Delta H"  
 /db\_xref="taxon:187420"  
 /clone="MTH"  
 /note="synonym: Methanobacterium thermoautotrophicum str.  
 deltaH"  
 ....  
 gene 1060742..1061974  
 /gene="MTH1150"  
 CDS 1060742..1061974  
 /gene="MTH1150"  
 /note="Function Code:12.02 - Cell Processes, Transport of  
 carbohydrates organic acids alcohols and lipids ; similar  
 to, gp:GI:g726070 LN:MTU19364, p()=1.1E-203, pid=93%"  
 /codon\_start=1  
 /transl\_table=11  
 /product="ABC transporter subunit Ycf24"  
 /protein\_id="NP\_276278.1"  
 /db\_xref="GI:15679161"  
 /db\_xref="CDD:pfam01458"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```
/db_xref="COG:COG0719"  
/translation="MLRDTLKKAEKAREKKALYGEDIDLEKFIKEEAGEHEEVTRAKE  
VPKEVQETLLRVGVDPEERERAGTFIQVDQSGICTTCASESIEIMGMNVALDKYSWLK  
DYMWKAVAVDTDKYTATTALREAEGEMGGYFIRSKPGAREVFPLQACMFIGDERVMQT  
AHNIVIAEENSELHIITGCATGEDVSSALHVGVSFYLLKKGARITFTMVHNWAEQVEV  
RPTGIMVGDDATYINNYILTSVPKSIQSYPTAYCTGENSRVVFQSILGGQKDSVLDL  
GSRVILEGRGSSAEMVSRVSKDSSQIYSRGLAGRVPEVKHLECHGLVLSDDSMIY  
AVPELEGSATELEMSHEAAVGKIAEEVMYLTSRGLTEEEAASMIVRGFLSMDITGLP  
PELAAETKRMLDMSLKGM"
```



# APPENDIX D

## Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

6: NC\_001799. *Toxoplasma gondii*...[gi:11496534]

### Links

LOCUS	NC_001799	34996 bp	DNA	circular INV 19-SEP-2002
DEFINITION	<i>Toxoplasma gondii</i> apicoplast, complete genome.			
ACCESSION	NC_001799			
VERSION	NC_001799.1 GI:11496534			
KEYWORDS	.			
SOURCE	apicoplast <i>Toxoplasma gondii</i>			
ORGANISM	<i>Toxoplasma gondii</i> Eukaryota; Alveolata; Apicomplexa; Coccidia; Eimeriida; Sarcocystidae; <i>Toxoplasma</i> .			
REFERENCE	1 (bases 4337 to 4925)			
AUTHORS	Beckers,C.J., Roos,D.S., Donald,R.G., Luft,B.J., Schwab,J.C., Cao,Y. and Joiner,K.A.			
TITLE	Inhibition of cytoplasmic and organellar protein synthesis in <i>Toxoplasma gondii</i> . Implications for the target of macrolide antibiotics			
JOURNAL	Journal of Clin. Invest. 95, 367-376 (1995)			
REFERENCE	2 (bases 13791 to 14996)			
AUTHORS	Kohler,S., Delwiche,C.F., Denny,P.W., Tilney,L.G., Webster,P., Wilson,R.J., Palmer,J.D. and Roos,D.S.			
TITLE	A plastid of probable green algal origin in Apicomplexan parasites			
JOURNAL	Science 275 (5305), 1485-1489 (1997)			
MEDLINE	97197911			
PUBMED	9045615			
REFERENCE	3 (bases 13218 to 15870; 28669 to 34697)			
AUTHORS	Denny,P., Preiser,P., Williamson,D. and Wilson,I.			
TITLE	Evidence for a single origin of the 35 kb plastid DNA in Apicomplexans			
JOURNAL	Protist 149, 51-59 (1998)			
REFERENCE	4 (bases 1 to 34996)			
AUTHORS	Kissinger,J.C., Donald,R.G., Moulton,A.L., Gutell,R., Aiello,D.P., Lang-Unnasch,N. and Roos,D.S.			
TITLE	Mapping, cloning, and complete sequence annotation of the 35-kb plastid genome of <i>Toxoplasma gondii</i>			
JOURNAL	Unpublished			
REFERENCE	5 (bases 1 to 34996)			
AUTHORS	Kissinger,J.C., Donald,R.G., Moulton,A.L., Aiello,D.P., Lang-Unnasch,N. and Roos,D.S.			
TITLE	Direct Submission			
JOURNAL	Submitted (16-JAN-1997) Biology, University of Pennsylvania, 415 S. University Ave, Philadelphia, PA 19104, USA			
REFERENCE	6 (bases 1 to 34996)			
AUTHORS	Kissinger,J.C., Donald,R.G., Moulton,A.L., Aiello,D.P., Lang-Unnasch,N. and Roos,D.S.			
TITLE	Direct Submission			
JOURNAL	Submitted (28-JUN-1999) Biology, University of Pennsylvania, 415 S. University Ave, Philadelphia, PA 19104, USA			
REMARK	Sequence update by submitter			
COMMENT	REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U87145. The <i>T. gondii</i> apicoplast genome exhibits non-standard codon usage: 33 in-frame UGA codons interrupt 17 of the 28 predicted coding regions, and are presumed to encode tryptophan. In addition, two genes ( <i>rps8</i> and <i>rpoC2</i> ) contain in-frame UAA or UAG stop codons which are currently being investigated further.			

**APPENDIX D**  
**Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database**

```
...
CDS      complement(28289..29686)
         /note="ycf24 homolog; in frame UGA codon predicted to
         encode tryptophan; similar to Plasmodium falciparum
         plastid genome ORF470"
         /codon_start=1
         /transl_except=(pos:complement(28916..28918),aa:Trp)
         /transl_table=11
         /product="ABC transporter"
         /protein_id="NP_044570.1"
         /db_xref="GI:11496556"
         /translation="MKLYKYLYNKYNNNTDLFNTVRLIGGLNINMVNKLIFKQDNFIF
         LYIFRLNALSILNKFKQPDWCFYELPEFAFDDISYYSIPLNVYTNKNKYKSIILSKLGL
         ELKFSENILLDVIFDSVLLNLTTFFLIKMGFLFSLFFQSIIFYYPYLIFSGLSIVSN
         TDNFFLTINSIIFNEGSFCFVMKDLNSNINLTTYFRTHSENFAQFERTLIVLSENSKL
         IYFEGCSAPMFLESQFHIAIVELFIKTKANLKYSTIQNWYRGNQLGEGGLYNFTTKRG
         FCMDKSFLNWIQIEIGSVITWKYPSTYLIGNKSFSNFFSLAMLSQVSDTGTKMLHI
         GKNTKSFILSKSLSFNFSFYTYRGLVTIFKTALNSYNYTECNLLIGCNAFTATIPYT
         IINNFSAYINQEATISKLELDFLFFLLHRGLNLKSTLMILIYGYCYNICKISFELEL
         EVPLLIIVARAQKLFY"
```

# **APPENDIX D** **Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

7: NC\_001713. *Odontella sinensis*...[gi:11467432]

## Links

```

LOCUS      NC_001713      119704 bp      DNA      circular PLN 19-SEP-2002
DEFINITION Odontella sinensis chloroplast, complete genome.
ACCESSION  NC_001713
VERSION    NC_001713.1  GI:11467432
KEYWORDS   .
SOURCE     chloroplast Odontella sinensis
  ORGANISM Odontella sinensis
            Eukaryota; stramenopiles; Bacillariophyta; Coscinodiscophyceae;
            Biddulphiophycidae; Eupodiscales; Eupodiscaceae; Odontella.
REFERENCE  1 (bases 1 to 119704)
  AUTHORS  Kowallik,K.V., Stoebe,B., Schaffran,I., Kroth-Pancic,P. and
            Freier,U.
  TITLE    The Chloroplast Genome of a chlorophyll a+c- containing Alga,
            Odontella sinensis
  JOURNAL  Plant Mol. Biol. Rep. 13, 336-342 (1995)
REFERENCE  2 (bases 1 to 119704)
  AUTHORS  Kowallik,K.V.
  TITLE    Direct Submission
  JOURNAL  Submitted (10-NOV-1995) Heinrich-Heine Universitaet Duesseldorf,
            Universitaetsstr. 1 Geb.26 13/02/46, Duesseldorf D-40225, Germany
COMMENT    REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from Z67753.
FEATURES   Location/Qualifiers
     source          1..119704
                     /organism="Odontella sinensis"
                     /organelle="plastid:chloroplast"
                     /db_xref="taxon:2839"

...
gene          69086..70546
               /gene="ycf24"
CDS           69086..70546
               /gene="ycf24"
               /note="ORF486"
               /codon_start=1
               /transl_table=11
               /product="ABC transporter"
               /protein_id="NP_043655.1"
               /db_xref="GI:11467509"
               /db_xref="SWISS-PROT:P49530"
               /translation="MTNKSNIKILNTNITKLVNQPYKYGFSTVIEKDIIIEKGLNEDVIC
LISKKKNEPKFLLEFRLKAFKKWKEMKCPDWAQIKFSEIDYQDIYYISAPKVKKKLNS
LDEVDPPELLKTFEKLGISLTEQKRLANVAIDAVFDSVSIATTFKEELAECGVIFSSIS
EAIQEYPELIEKYLGSVVPIGDNYFSALNSAVFTDGSFCYIPKDTICPLELSTYFRIN
DQKSGQFERTLIVAEKNSQVSYLEGCTAPQYDSNQLHAAVVELVALENADIKYSTVQN
WYAGNNYGEggiYNFVTKRGLCAGSNSKISWTQVETGSNITWKYPSCLLVGDKAKGEF
YSVALTNNYQQADTGSKMIHVGNTRSRIVSKGISAGNSKNTYRGLVNISNKAIGARN
YSQCDSLLIGNLSNANTFPFISVQNPTAKIEHEASTSKIGEEQIFYFLQRGIPIEKGV
ELMISGFCQEVFTELPLEFAAEADRLTLKLEGSVG"

```

# APPENDIX D

## Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

8: NC\_001675. Cyanophora parado... [gi:11467282]

### Links

LOCUS NC\_001675 135599 bp DNA circular PLN 19-SEP-2002  
 DEFINITION Cyanophora paradoxa cyanelle, complete genome.  
 ACCESSION NC\_001675  
 VERSION NC\_001675.1 GI:11467282  
 KEYWORDS .  
 SOURCE cyanelle Cyanophora paradoxa  
 ORGANISM Cyanophora paradoxa  
 Eukaryota; Glaucocystophyceae; Cyanophoraceae; Cyanophora.  
 REFERENCE 1 (bases 1 to 135599)  
 AUTHORS Stirewalt,V.L., Michalowski,C.B., Luffelhardt,W., Bohnert,H.J. and Bryant,D.A.  
 TITLE Nucleotide sequence of the cyanelle genome from Cyanophora paradoxa  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 135599)  
 AUTHORS Bryant,D.A.  
 TITLE Direct Submission  
 JOURNAL Submitted (01-JUL-1995) Donald A. Bryant, Biochemistry and Molecular Biology, The Pennsylvania State University, S-234 Freear Bldg., University Park, PA 16802, USA  
 COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U30821.  
 FEATURES  
 source Location/Qualifiers  
 1..135599  
 /organism="Cyanophora paradoxa"  
 /organelle="plastid:cyanelle"  
 /strain="Pringsheim LB555"  
 /db\_xref="taxon:2762"  
 ...  
 gene complement (67893..69353)  
 /gene="ycf24"  
 CDS complement (67893..69353)  
 /gene="ycf24"  
 /codon\_start=1  
 /transl\_table=11  
 /product="ABC transporter"  
 /protein\_id="NP\_043219.1"  
 /db\_xref="GI:11467362"  
 /translation="MVNTQSPKNSGLENLVNQPYKYGLPLIFEIETISKGLTEETIRL  
 ISEKKNEPQFMLEFRLQAYRKWLEMSNEPEWAHLNYPKINYQDMVYYSAPKQKKKLQS  
 LDEVDP TLLETFEKLGIP LTEQKRLANVAVD AIFDSVSVATT FKEELAKEGVIFCPIS  
 EAVQKYPDLIKKYLGSVVSTSDNYFSCLNAAVFS DGSFCYIPKNVRCPLELSTYFRIN  
 NGESGQFERTLIVADEGSYVSYLEGCTAPQFD TNQLHAAVVELVALDNAEI KYSTVQN  
 WYAGDENGKGGIYNFVTKRGLCAGKNSKISWTQVETGSAITWKYPSCVLLGDNSIGEF  
 YSVALTNRYQQADTGKMIHIGKNTRSRIISKGISAGHSQNSYRGLVKIGPKAVGARN  
 YSQCD SLLIGDNSQANTFPHLQIKNPTAKVEHEASTSKIGEEQIFYFLQRGINAE EAI  
 SLIISGFCREVFNNLPMEFALEADKLLGLKLEGSVG"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

9: NC\_000925. *Porphyra purpurea*...[gi:11465652]

#### Links

```

LOCUS      NC_000925      191028 bp      DNA      circular PLN 27-AUG-2002
DEFINITION Porphyra purpurea chloroplast, complete genome.
ACCESSION  NC_000925
VERSION    NC_000925.1  GI:11465652
KEYWORDS   .
SOURCE     chloroplast Porphyra purpurea
  ORGANISM Porphyra purpurea
            Eukaryota; Rhodophyta; Bangiophyceae; Bangiales; Bangiaceae;
            Porphyra.
REFERENCE  1 (bases 1 to 191028)
  AUTHORS  Reith,M.E. and Munholland,J.
  TITLE    Complete nucleotide sequence of the Porphyra purpurea chloroplast
            genome
  JOURNAL  Plant Mol. Biol. Rep. 13 (4), 333-335 (1995)
REFERENCE  2 (bases 1 to 191028)
  AUTHORS  Reith,M.E.
  TITLE    Direct Submission
  JOURNAL  Submitted (17-OCT-1995) Michael E. Reith, Marine Biology Section,
            NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax,
            Nova Scotia B3H 3Z1, Canada
COMMENT    REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from U38804.
FEATURES   Location/Qualifiers
            source          1..191028
                               /organism="Porphyra purpurea"
                               /organelle="plastid:chloroplast"
                               /strain="Avonport"
                               /db_xref="taxon:2787"

```

...

```

gene       40948..42411
            /gene="ycf24"
CDS        40948..42411
            /gene="ycf24"
            /note="hypothetical chloroplast ORF 24"
            /codon_start=1
            /transl_table=11
            /product="ABC transporter"
            /protein_id="NP_053850.1"
            /db_xref="GI:11465706"
            /translation="MVNTQNQISQTSDDLDIYNQPYKYGFTTSVESEQFPRGISREVV
            KLISKKKNEPEYLLNFRLLKAYEKWTKMKNPKWAHLKHPNIDFNSIIYYAVPKLKKELN
            SLDEVDP EILDTFNKLGISLNEQKRLSNVAVDAVFDSVSIATTFKKELAEAGVIFCSI
            SEAIRNYPDLIQYLGTVVPSGDNYFAALNSAVFSDGSFCYIPDPTVCPLELSTYFRI
            NNEESGQFERTLIVADRGSKVSYLEGCTAPQYDTNQLHAAIVELIALDDAEIKYSTVQ
            NWYAGNKDGKGGIYNFVTKRGLCSGKNSKISWTQVETGSAITWKYPGCILAGDNSQGE
            FYSVALTNNYQEADTGTKMIHIGNNTKSKIISKGISAGKSKNSYRGLVKIGPQSFNSR
            NYSQCDSLLIGQSSQANTFPYIQVQNPTAKVEHEASTKISEDQIFYFLQRGINLEES
            VSLMISGFCKDVFNELPMEFAVEADRLLSLKLEGTVG"

```

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

10: NC\_000926. *Guillardia theta* ...[gi:11467607]

### Links

LOCUS	NC_000926	121524 bp	DNA	circular	PLN 22-AUG-2002
DEFINITION	<i>Guillardia theta</i> chloroplast, complete genome.				
ACCESSION	NC_000926				
VERSION	NC_000926.1 GI:11467607				
KEYWORDS	.				
SOURCE	chloroplast <i>Guillardia theta</i>				
ORGANISM	<i>Guillardia theta</i> Eukaryota; Cryptophyta; Cryptomonadaceae; <i>Guillardia</i> .				
REFERENCE	1 (bases 47701 to 48415)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	The small subunit of ribulose-1,5-bisphosphate carboxylase is plastid-encoded in the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	Plant Mol. Biol. 13 (1), 13-20 (1989)				
MEDLINE	93357429				
PUBMED	2562756				
REFERENCE	2 (bases 18535 to 19351)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	Sequence analysis of the plastid rDNA spacer region of the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	DNA Seq. 1 (1), 55-62 (1990)				
MEDLINE	92119320				
PUBMED	2132959				
REFERENCE	3 (bases 43739 to 44938)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	Nucleotide sequence of the genes for ribosomal protein S4 and tRNA(Arg) from the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	Nucleic Acids Res. 18 (7), 1903 (1990)				
MEDLINE	90245597				
PUBMED	2336372				
REFERENCE	4 (bases 34539 to 35380)				
AUTHORS	Reith,M. and Douglas,S.				
TITLE	Localization of beta-phycoerythrin to the thylakoid lumen of <i>Cryptomonas phi</i> does not involve a signal peptide				
JOURNAL	Plant Mol. Biol. 15 (4), 585-592 (1990)				
MEDLINE	91338697				
PUBMED	2102376				
REFERENCE	5 (bases 45872 to 47981)				
AUTHORS	Douglas,S.E., Durnford,D.G. and Morden,C.W.				
TITLE	Nucleotide sequence of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase from the chlorophyll c-containing Alga <i>Cryptomonas F</i> : evidence supporting the polyphyletic origin of plastids				
JOURNAL	J. Phycol. 26, 500-508 (1990)				
REFERENCE	6 (bases 110917 to 113854)				
AUTHORS	Douglas,S.E.				
TITLE	Unusual organization of a ribosomal protein operon in the plastid genome of <i>Cryptomonas phi</i> : evolutionary considerations				
JOURNAL	Curr. Genet. 19 (4), 289-294 (1991)				
MEDLINE	91330343				
PUBMED	1868578				
REFERENCE	7 (bases 40675 to 42376)				
AUTHORS	Douglas,S.E. and Turner,S.				
TITLE	Molecular evidence for the origin of plastids from a				

## APPENDIX D

Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

cyanobacterium-like ancestor

JOURNAL J. Mol. Evol. 33 (3), 267-273 (1991)

MEDLINE 92099311

PUBMED 1757997

REFERENCE 8 (bases 96129 to 98906)

AUTHORS Wang, S.L. and Liu, X.Q.

TITLE The plastid genome of *Cryptomonas phi* encodes an hsp70-like protein, a histone-like protein, and an acyl carrier protein

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88 (23), 10783-10787 (1991)

MEDLINE 92073372

PUBMED 1961745

REFERENCE 9 (bases 106789 to 108216)

AUTHORS Douglas, S.E.

TITLE A *secY* homologue is found in the plastid genome of *Cryptomonas phi*

JOURNAL FEBS Lett. 298 (1), 93-96 (1992)

MEDLINE 92183838

PUBMED 1544427

REFERENCE 10 (bases 42198 to 44153)

AUTHORS Douglas, S.E. and Reith, M.E.

TITLE A *bchI* homolog, encoding a subunit of Mg chelatase, is located on the plastid genomes of red and cryptomonad algae

JOURNAL J. Mar. Biotechnol. 1, 135-141 (1993)

REFERENCE 11 (bases 82327 to 84479)

AUTHORS Douglas, S.E. and Murphy, C.A.

TITLE Structural, transcriptional and phylogenetic analyses of the *atpB* gene cluster from the plastid of *Cryptomonas F* (Cryptophyceae)

JOURNAL J. Phycol. 30, 329-340 (1994)

REFERENCE 12 (bases 98901 to 114602)

AUTHORS Wang, S.L., Liu, X.Q. and Douglas, S.E.

TITLE The large ribosomal protein gene cluster of a cryptomonad plastid: gene organization, sequence and evolutionary implications

JOURNAL Biochem. Mol. Biol. Int. 41 (5), 1035-1044 (1997)

MEDLINE 97283757

PUBMED 9137835

REFERENCE 13 (bases 61067 to 68605)

AUTHORS Leitsch, C.E.W., Kowallik, K.V. and Douglas, S.E.

TITLE The *atpA* gene cluster of a cryptomonad, *Guillardia theta*: A piece in the puzzle of chloroplast genome development

JOURNAL J. Phycol. (1998) In press

REFERENCE 14 (bases 1 to 121524)

AUTHORS Douglas, S.E. and Penny, S.L.

TITLE The plastid genome of the cryptophyte alga, *Guillardia theta*: complete sequence and conserved syntenic groups confirm its common ancestry with red algae

JOURNAL J. Mol. Evol. 48 (2), 236-244 (1999)

MEDLINE 99128221

PUBMED 9929392

REFERENCE 15 (bases 1 to 121524)

AUTHORS Douglas, S.E.

TITLE Direct Submission

JOURNAL Submitted (08-JAN-1998) Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada

COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from AF041468.

FEATURES

source Location/Qualifiers

1..121524

/organism="Guillardia theta"

/organelle="plastid:chloroplast"

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

```
/db_xref="taxon:55529"
/note="plastid"
```

...

```
gene      61067..62518
           /gene="ycf24"
CDS       61067..62518
           /gene="ycf24"
           /note="hypothetical chloroplast RF24"
           /codon_start=1
           /transl_table=11
           /product="ABC transporter"
           /protein_id="NP_050730.1"
           /db_xref="GI:11467678"
           /translation="MSDDLKRSRLRELVSQPYKYGFHTDIENEEFPKGLDEDI I KEIS
KLKCEPSYMLDFRLKSYILWKKMSLPEWACLTYLNINYQDIVYYSAPKNSTKLD SLED
VDKKILETFDKLGIPLNEQKKLANVAVD AIFDSVSVGTTFKQELSNVGVLCPLSEAT
NKYSTLVEKYLGSVVP IGDNYFAALNSAVFSEGSFCYIPPNVKCPLELSTYFRINNEN
SGQFERTLI IADFNSYVSYLEGCTAPMYDKNQLHAAVVELIALENAEIRYSTVQNWYS
GDTNGKGGIYNFVTKRGLCAGKSSKISWTQVETGSAITWKYPSCILVGEDSVGEFY SV
ALTNNYQQADTGTKMIHVGRGSKSRIISKGISAGYSKNTYRGQVKININALGSINNSQ
CDSMLIGPYSQANTYPYIQVSNAMSRVEHEASTSKIEEEQLFYFLQRGISVEQAISLL
ISGFCRDV FVKLPMEFAVEADKLLSVKLEGTVG"
```



## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

11:NC\_001840. Cyanidium caldari...[gi:11465393]

#### Links

```

LOCUS      NC_001840      164921 bp      DNA      circular PLN 22-AUG-2002
DEFINITION Cyanidium caldarium chloroplast, complete genome.
ACCESSION  NC_001840
VERSION    NC_001840.1  GI:11465393
KEYWORDS   .
SOURCE     chloroplast Cyanidium caldarium
  ORGANISM Cyanidium caldarium
            Eukaryota; Rhodophyta; Bangiophyceae; Porphyridiales;
            Porphyridiaceae; Cyanidium.
REFERENCE  1 (bases 130696 to 132364)
  AUTHORS  Vogel,H., Fischer,S. and Valentin,K.
  TITLE    A model for the evolution of the plastid sec apparatus inferred
            from secY gene phylogeny
  JOURNAL  Plant Mol. Biol. 32 (4), 685-692 (1996)
  MEDLINE  97134960
  PUBMED   8980520
REFERENCE  2 (bases 1 to 164921)
  AUTHORS  Glockner,G., Rosenthal,A. and Valentin,K.
  TITLE    The structure and gene repertoire of an ancient red algal plastid
            genome
  JOURNAL  J. Mol. Evol. 51 (4), 382-390 (2000)
  MEDLINE  20496959
  PUBMED   11040290
REFERENCE  3 (bases 46857 to 47851)
  AUTHORS  Valentin,K.
  TITLE    Direct Submission
  JOURNAL  Submitted (22-MAR-1996) Institute for Plant Physiology, Justus
            Liebig University, Heinrich Buff Ring 58-62, Giessen 35392, Germany
REFERENCE  4 (bases 28701 to 75580)
  AUTHORS  Gloeckner,G., Rosenthal,A. and Valentin,K.
  TITLE    Direct Submission
  JOURNAL  Submitted (02-SEP-1997) Department of Genome Analysis, IMB Jena,
            Beutenbergstr.11, Jena 07745, Germany
REFERENCE  5 (bases 1 to 164921)
  AUTHORS  Gloeckner,G., Rosenthal,A. and Valentin,K.
  TITLE    Direct Submission
  JOURNAL  Submitted (18-NOV-1999) Genome Analysis, Institute for Molecular
            Biotechnology, Beutenbergstrasse 11, Jena 07745, Germany
REFERENCE  6 (bases 130696 to 132364)
  AUTHORS  Vogel,H., Fischer,S. and Valentin,K.
  TITLE    Direct Submission
  JOURNAL  Submitted (18-NOV-1999) Institute for Plant Physiology, Justus
            Liebig University, Heinrich Buff Ring 58-62, Giessen 35392, Germany
COMMENT    REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from AF022186.
FEATURES   Location/Qualifiers
    source   1..164921
             /organism="Cyanidium caldarium"
             /organelle="plastid:chloroplast"
             /strain="RK1"
             /db_xref="taxon:2771"
    repeat_region join(164808..164921,1..78,462..644)
             /note="repeat unit separated by stem loop"
             /rpt_type=direct

```

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### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```

stem_loop      339..477
                /note="stem-loop separates direct repeat unit"
rep_origin     580..780
                /note="similiar to yeast mitochondrial origin of
                replication region in GenBank Accession Number L36902"
...
gene           114386..115837
                /gene="ycf24"
CDS            114386..115837
                /gene="ycf24"
                /note="similar to ycf24 in Porphyra purpurea"
                /codon_start=1
                /transl_table=11
                /product="ABC transporter"
                /protein_id="NP_045146.1"
                /db_xref="GI:11465463"
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                HKKQEPDFILKFRLKAYQIWKMMQAPDWGHLHHNEINFNDVLCYASPKLEQGKNAQT
                ISEEILATFEKLGVPKPKNNKQPKIAVDAVFDSISFGTTLQKELKEQGIIFCSISEAI
                KAYPNLIKLYLGSIVPAGDNYFAALNSAVFTDGSFCYIPKNIRCPVDLSTYFRINNKE
                AGQFERTLIIADENSFVNYLEGCTAPQFDTNQLHAAVVELICFKNATINYSTVQNWYA
                GNNKGEQGVYNFVTKRGLCQGENSKISWTQLETGSAITWKYPSCLLKGKRSTGEFFSV
                TLTNNAQEADTGTKMLHFGRQSKSLVISKGISGGVSKNTYRGLVKISGSAIYSDNRSQ
                CDSLLIGKGSSENTYPNLHVHNSLSKVEHEAFVSRIGEEQIFYFQQRGINIEEALNMI
                VSGFCQDVCNKLPMFALEANKLLNIKLEGSIG"

```

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## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

12: AP005370. Thermosynechococc... [gi:22294033]

### Links

LOCUS AP005370 299350 bp DNA linear BCT 17-AUG-2002  
DEFINITION Thermosynechococcus elongatus BP-1 DNA, complete genome, section 2/9.  
ACCESSION AP005370 BA000039  
VERSION AP005370.1 GI:22294033  
KEYWORDS .  
SOURCE Thermosynechococcus elongatus BP-1  
ORGANISM Thermosynechococcus elongatus BP-1  
Bacteria; Cyanobacteria; Chroococcales; Thermosynechococcus.  
REFERENCE 1  
AUTHORS Nakamura,Y., Kaneko,T., Sato,S., Ikeuchi,M., Katoh,H., Sasamoto,S., Watanabe,A., Iriguchi,M., Kawashima,K., Kimura,T., Kishida,Y., Kiyokawa,C., Kohara,M., Matsumoto,M., Matsuno,A., Nakazaki,N., Shimpo,S., Sugimoto,M., Takeuchi,C., Yamada,M. and Tabata,S.  
TITLE Complete genome structure of the thermophilic cyanobacterium Thermosynechococcus elongatus BP-1  
JOURNAL DNA Res. (2002) In press  
REFERENCE 2 (bases 1 to 299350)  
AUTHORS Kaneko,T.  
TITLE Direct Submission  
JOURNAL Submitted (05-JUN-2002) Takakazu Kaneko, Kazusa DNA Research Institute, The First Laboratory for Plant Gene Research; 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0812, Japan (E-mail:kaneko@kazusa.or.jp, URL:http://www.kazusa.or.jp/cyano/Thermo/, Tel:81-438-52-3935(ex.2338), Fax:81-438-52-3934)  
FEATURES  
source Location/Qualifiers  
1..299350  
/organism="Thermosynechococcus elongatus BP-1"  
/strain="BP-1"  
/db\_xref="taxon:197221"  
/note="BP-1"  
...  
gene complement(185829..187265)  
/gene="ycf24"  
CDS complement(185829..187265)  
/gene="ycf24"  
/note="ORF\_ID:t110490"  
/codon\_start=1  
/transl\_table=11  
/product="ABC transporter subunit"  
/protein\_id="BAC08042.1"  
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PEFMLEFRLRAYRQWLKMSEPQWPRVSYPINQDIVYYSAPKQKEKLKSLDEVDPVL  
LETFEKLGIPLSEQKRLTNVAVD AIFDSVSVATTFREEELAKQGIIFCSISEALQDYPE  
LVQKYLGSVVP IGDNFYAALNSAVFSDGSFVYVPKNTRCPMELSTYFRINNGESGQFE  
RTLIIADAGSYVSYLEGCTAPMFDTNQLHAAVVELVALDNAEIKYSTVQNWYAGDENG  
KGGIYNFVTKRGLCLGRNSKISWTQVETGSAITWKYPSCVLVGDNSVGEFYVALTNH  
YQQADTGTKMIHIGKNTRSRIVSKGISAGHSQNSYRGLVKIGPKATGARNYSQCDSML  
IGDTAAANTFPYIQVQNPTAQVEHEASTSKIGEDQLFYFAQRGISAEDAVSMMISGFC  
RDVFNQLPMEFAVEADRLLSLKLEGSVG"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

13:AE000884. Methanobacterium ...[gi:2622242]

#### Links

LOCUS	AE000884	11204 bp	DNA	linear	BCT 19-JUN-2002
DEFINITION	Methanobacterium thermoautotrophicum from bases 1050856 to 1062059 (section 90 of 148) of the complete genome.				
ACCESSION	AE000884 AE000666				
VERSION	AE000884.1 GI:2622242				
KEYWORDS	.				
SOURCE	Methanothermobacter thermautotrophicus str. Delta H (Methanobacterium thermoautotrophicum str. deltaH)				
ORGANISM	Methanothermobacter thermautotrophicus str. Delta H Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanothermobacter.				
REFERENCE	1 (bases 1 to 11204)				
AUTHORS	Smith,D.R., Doucette-Stamm,L.A., Deloughery,C., Lee,H., Dubois,J., Aldredge,T., Bashirzadeh,R., Blakely,D., Cook,R., Gilbert,K., Harrison,D., Hoang,L., Keagle,P., Lumm,W., Pothier,B., Qiu,D., Spadafora,R., Vicaire,R., Wang,Y., Wierzbowski,J., Gibson,R., Jiwani,N., Caruso,A., Bush,D. and Reeve,J.N.				
TITLE	Complete genome sequence of Methanobacterium thermoautotrophicum deltaH: functional analysis and comparative genomics				
JOURNAL	J. Bacteriol. 179 (22), 7135-7155 (1997)				
MEDLINE	98037514				
PUBMED	9371463				
REFERENCE	2 (bases 1 to 11204)				
AUTHORS	Smith,D.R.				
TITLE	Direct Submission				
JOURNAL	Submitted (10-AUG-1997) Genomics and Technology Development, Genome Therapeutics Corporation, 100 Beaver Street, Waltham, MA 02154-8448, USA				
FEATURES	Location/Qualifiers				
source	1..11204 /organism="Methanothermobacter thermautotrophicus str. Delta H" /strain="Delta H" /db_xref="taxon:187420" /clone="MTH" /note="synonym: Methanobacterium thermoautotrophicum str. deltaH"				
...					
gene	9887..11119 /gene="MTH1150"				
CDS	9887..11119 /gene="MTH1150" /note="Function Code:12.02 - Cell Processes, Transport of carbohydrates organic acids alcohols and lipids ; similar to, gp:GI:g726070 LN:MTU19364, p()=1.1E-203, pid=93%" /codon_start=1 /transl_table=11 /product="ABC transporter subunit Ycf24" /protein_id="AAB85639.1" /db_xref="GI:2622255" /translation="MLRDTLKKAEKAREKKALYGEDIDLEKFIKEEAGEHEEVTRAKE VPKEVQETLLRVGVDPEERERAGTFIQVDQSGICTTCASESIEIMGMNVALDKYSWLK DYMWKAVAVDTDKYTATTALREAEGEMGGYFIRSKPGAREVFPLQACMFIGDERVMQT"				

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### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

AHNIVIAEENSELHIITGCATGEDVSSALHVGVSEFYLLKKGARITFTMVHNWAEQVEV  
RPRTGIMVGDDATYINNYILTSVPKSIQSYPTAYCTGENSRVVFQSIILGGQKDSVLDLDM  
GSRVILEGRGSSAEMVSRAVSKDSSQIYSRGHLAGRVPEVKGHLECHGLVLSDDSMIY  
AVPELEGSATELEMSHEAAVGKIAEEVMYLTSRGLTEEEAASMIVRGFLSMDITGLP  
PELAAETKRMLDMSLKGM"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

14: AP003589. *Nostoc* sp. PCC 71...[gi:17131372]

#### Links

LOCUS	AP003589	341880 bp	DNA	linear	BCT 28-NOV-2001
DEFINITION	Nostoc sp. PCC 7120 DNA, complete genome, section 9/19.				
ACCESSION	AP003589 BA000019				
VERSION	AP003589.1 GI:17131372				
KEYWORDS	.				
SOURCE	Nostoc sp. PCC 7120				
ORGANISM	Nostoc sp. PCC 7120				
	Bacteria; Cyanobacteria; Nostocales; Nostocaceae; Nostoc.				
REFERENCE	1				
AUTHORS	Kaneko,T., Nakamura,Y., Wolk,C.P., Kuritz,T., Sasamoto,S., Watanabe,A., Iriguchi,M., Ishikawa,A., Kawashima,K., Kimura,T., Kishida,Y., Kohara,M., Matsumoto,M., Matsuno,A., Muraki,A., Nakazaki,N., Shimpo,S., Sugimoto,M., Takazawa,M., Yamada,M., Yasuda,M. and Tabata,S.				
TITLE	Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium <i>Anabaena</i> sp. strain PCC 7120				
JOURNAL	DNA Res. 8 (5), 205-213 (2001)				
MEDLINE	21595285				
PUBMED	11759840				
REFERENCE	2 (bases 1 to 341880)				
AUTHORS	Kaneko,T.				
TITLE	Direct Submission				
JOURNAL	Submitted (02-MAY-2001) Takakazu Kaneko, Kazusa DNA Research Institute, The First Laboratory for Plant Gene Research; Yana 1532-3, Kisarazu, Chiba 292-0812, Japan (E-mail:kaneko@kazusa.or.jp, URL:http://www.kazusa.or.jp/cyanobase/, Tel:81-438-52-3935(ex.2338), Fax:81-438-52-3934)				
FEATURES	Location/Qualifiers				
source	1..341880 /organism="Nostoc sp. PCC 7120" /db_xref="taxon:103690" /note="synonym:Anabaena sp. PCC7120"				
...					
gene	244219..245658 /gene="alr2492"				
CDS	244219..245658 /gene="alr2492" /note="ORF_ID:alr2492 probable ABC transporter membrane protein ( <i>ycf24</i> )" /codon_start=1 /transl_table=11 /protein_id="BAB74191.1" /db_xref="GI:17131584" /translation="MSATVKTLVNQPYKYGFVTDIEADTI PRGLDEDVVRLISTKKNE PEFMLEFRLRAFRQWQKMTPTWPSVKYPPIDYQNI IYYSAPKQKKAKLNSLDEVDP LIETFEKLGIPLSEQKRLANVAVD AIFDSVSVATT FKEKLAKDGVIFCSI SEALQEH ELIKKYLGSVVPIADNYFAALNAAVFSDGSFVYIPKGVKCPMELSTYFRINSGDTGQF ERTLIVAEESVYSYLEGCTAPMYDSNQLHAAVVELVALDNAEIKYSTVQNWYAGDAN GKGGIYNFVTKRGLCQGVNSKISWTQVETGSAITWKYPSCVLVGDNSVGEFYSVALTN NMQQADTGTKMIHIGKNRSTIISKGISAGQSSNSYRGLVKINPTAKGARNYSQCDSM LIGDNAHANTFPYIQVQNN TGKVEHEASTSKIGEDQLFFFAQRGISSEDAISMMISGF CKDVFNQLPMEFAVEADKLLSLKLEGSVG"				

**APPENDIX D**  
**Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database**

15: BI437350. gc59d11.y1 Moss E...[gi:15262040]

Links

IDENTIFIERS

dbEST Id: 9279936  
EST name: gc59d11.y1  
GenBank Acc: BI437350  
GenBank gi: 15262040

CLONE INFO

Clone Id: PEP\_SOURCE\_ID:PPN200721 (5')  
Source: University of Leeds (UK) & Washington University in St. Louis (USA)  
DNA type: cDNA

PRIMERS

PolyA Tail: Unknown

SEQUENCE

GGGAAGTCTTTAAGGAGCTTCCTCTGGAGTTTGCTGCTGAGGTCAATCAATTGATGAGCT  
TGAAGCTGGAGGGAAGCGTTGGCTAATTTATTTGTACCCAAGGTGTCGTTTCAAGAGATG  
TAGACGACGCTTGTTGTCAAATGCTTATGTCCTAGTTGGAGAATGCTTCGGTCCTGCTTA  
TCAAGATGTCTCCTGCTTTGTTTAAACCAAGGTTTCGAGTTCATGTATTTTAACATTTT  
ATATTCACACGCCACCAATGTCGGAAGTGAGGGAACTGATTGCGAAGAGGGAATAGATA  
TATACAATTCATGCGGTGCGCGGTGGATGAATTCAACTTTTCACATATGCCTCAGCTGT

Entry Created: Aug 21 2001  
Last Updated: Aug 21 2001

COMMENTS

Libraries were constructed by Dr. Stavros Bashiardes as part of the Physcomitrella EST program (PEP) at the Univ. of Leeds (UK) and Washington Univ. in St. Louis (USA) DNA sequencing by: Washington University Genome Sequencing Center For information on obtaining a clone please contact: Celia Knight (c.d.knight@leeds.ac.uk)

PUTATIVE ID Assigned by submitter  
SW:YC24\_ODOSI P49530 HYPOTHETICAL 54.3 KD PROTEIN YCF24 ;

LIBRARY

Lib Name: Moss EST library PPN  
Organism: Physcomitrella patens  
Tissue type: protonemata: 7 day old tissue auxin treated  
Lab host: DH10B  
Vector: pBluescript SK-  
R. Site 1: EcoRI  
R. Site 2: XhoI  
Description: Construction of the cDNA library was carried out using Stratagenes 'UniZAP - cDNA synthesis kit'. cDNA was constructed using an oligo dT primer/linker that contains a XhoI site within it. Following ds cDNA synthesis, EcoRI adapters were ligated to the blunt ends and sample was digested with XhoI. The result is cDNA with an EcoRI sticky end on one side and a XhoI sticky end on the other. This cDNA was ligated directionally in UniZAP arms. The vector is

**APPENDIX D**  
**Excerpts from 26 yc/24 gene product and gene sequences retrieved from NCBI database**

designed containing the pBluescript sequence as well as lambda DNA and cDNA is cloned within this pBluescript sequence. The vector was then packaged using Gold gigapackaging extracts. Library was grown in XL1Blue MRF<sup>+</sup> cells and amplified. The library was excised by mass excision using Stratagene's 'Mass excision kit' that uses exassist as a helper phage that releases the pBluescript sequence and circularises it as single stranded plasmids that are then packaged (by helper phage) and secreted out of the host cell as phagemids. SOLR cells were transformed with phagemids and the library was plated out on LB-amp plates to select for transformants. Approximately 1,000,000 colonies were grown and recovered. The double stranded plasmid library was recovered by using Quiagen Midi prep kit. 2 micro grams of each library were used to transform DH10B cells by electroporation.

**SUBMITTER**

Name: Ralph Quatrano  
Lab: Leeds/Wash U Moss EST Project  
Institution: Washington University School of Medicine  
Address: 4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA  
Tel: 314 286 1800  
Fax: 314 286 1810  
E-mail: est@watson.wustl.edu

**CITATIONS**

Title: Leeds/Wash U Moss EST Project  
Authors: Quatrano,R., Bashardes,S., Cove,D., Cuming,A., Knight,C., Clifton,S., Marra,M., Hillier,L., Pape,D., Martin,J., Wylie,T., Underwood,K., Theising,B., Allen,M., Bowers,Y., Person,B., Swaller,T., Steptoe,M., Gibbons,M., Harvey,N., Ritter,E., Jackson,Y., McCann,R., Waterston,R., Wilson,R.  
Year: 1999  
Status: Unpublished

**MAP DATA**



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**Excerpts from 26 yc/24 gene product and gene sequences retrieved from NCBI database**

16:1: Z67753. *Odontella sinensi*...[gi:1185127]

Links

LOCUS OSCHLPLXX 119704 bp DNA circular PLN 10-APR-2001  
DEFINITION *Odontella sinensis* complete chloroplast genome.  
ACCESSION Z67753  
VERSION Z67753.1 GI:1185127  
KEYWORDS 16S ribosomal RNA; 16S rRNA gene; 23S ribosomal RNA; 23S rRNA gene; 30S ribosomal protein S10; 30S ribosomal protein S11; 30S ribosomal protein S12; 30S ribosomal protein S13; 30S ribosomal protein S14; 30S ribosomal protein S16; 30S ribosomal protein S17; 30S ribosomal protein S18; 30S ribosomal protein S19; 30S ribosomal protein S2; 30S ribosomal protein S20; 30S ribosomal protein S3; 30S ribosomal protein S4; 30S ribosomal protein S5; 30S ribosomal protein S6; 30S ribosomal protein S7; 30S ribosomal protein S8; 30S ribosomal protein S9; 50S ribosomal protein L1; 50S ribosomal protein L11; 50S ribosomal protein L12; 50S ribosomal protein L13; 50S ribosomal protein L14; 50S ribosomal protein L16; 50S ribosomal protein L18; 50S ribosomal protein L19; 50S ribosomal protein L2; 50S ribosomal protein L20; 50S ribosomal protein L21; 50S ribosomal protein L22; 50S ribosomal protein L23; 50S ribosomal protein L24; 50S ribosomal protein L27; 50S ribosomal protein L29; 50S ribosomal protein L3; 50S ribosomal protein L31; 50S ribosomal protein L32; 50S ribosomal protein L33; 50S ribosomal protein L34; 50S ribosomal protein L35; 50S ribosomal protein L36; 50S ribosomal protein L4; 50S ribosomal protein L5; 50S ribosomal protein L6; 5S ribosomal RNA; 5S rRNA gene; acp gene; acyl carrier protein; apocytochrome f; ATP synthase CF1; ATP synthase CFO; atpA gene; atpB gene; atpD gene; atpE gene; atpF gene; atpG gene; atpH gene; atpI gene; cfxQ gene; chaperonin; chlI gene; clp protease; clpC gene; complete genome; CP43 chlorophyll apoprotein; CP47 chlorophyll apoprotein; cytochrome b559; cytochrome b6; cytochrome b6/f complex; cytochrome c550; D1 reaction-center protein; D2 reaction-center protein; DNA-replication helicase; dnaB gene; dnaK gene; elongation factor tu; Fe-S polypeptide; ferredoxin; ferredoxin-binding protein II; groEL gene; orf 179; ORF 312; ORF 644; ORF113; ORF128; ORF148; ORF181; ORF204; ORF25; ORF251; ORF263; ORF26a; ORF26b; ORF27; ORF29; ORF29a; ORF29b; ORF31; ORF319; ORF34; ORF355; ORF355 gene; ORF36; ORF380; ORF382; ORF41; ORF42; ORF44; ORF455; ORF46; ORF486; ORF497; ORF61; ORF64; ORF73; ORF74; ORF99; P700 apoprotein A1; P700 apoprotein A2; petA gene; petB gene; petD gene; petF gene; petG gene; petK gene; phosphoprotein; plastocyanin-binding; preprotein-translocase; protein I; protein J; protein K; protein L; protein M; protein N; protein T; protein W; protein X; psaA gene; psaB gene; psaC gene; psaD gene; psaE gene; psaF gene; psaI gene; psaJ gene; psal gene; psam gene; psbA gene; psbB gene; psbC gene; psbD gene; psbE gene; psbF gene; psbH gene; psbI gene; psbJ gene; psbK gene; psbL gene; psbN gene; psbT gene; psbV gene; psbW gene; psbX gene; PSI; PSII; rbcL gene; rbcR gene; rbcS gene; RNA polymerase; RNA polymerase b'-chain; RNA polymerase b'-chain; RNA polymerase b-chain; rpl1 gene; rpl11 gene; rpl12 gene; rpl13 gene; rpl14 gene; rpl16 gene; Rpl18 gene; rpl19 gene; rpl2 gene; rpl20 gene; rpl21 gene; rpl22 gene; rpl23 gene; rpl24 gene; rpl27 gene; rpl29 gene; rpl3 gene; rpl31 gene; rpl32 gene; rpl32' gene; rpl33 gene; rpl34 gene; rpl35 gene; rpl36 gene; rpl4 gene; rpl5 gene; rpl6 gene; rpoA gene; rpoB gene; rpoC1 gene; rpoC2 gene; rps10 gene; rps11 gene; rps12 gene; rps13 gene; rps14 gene; rps16 gene;

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### Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

rps17 gene; rps18 gene; rps19 gene; rps2 gene; rps20 gene; rps3 gene; rps4 gene; RPS5 gene; RPS6 gene; rps7 gene; rps8 gene; rps9 gene; rubisco large subunit; rubisco small subunit; secA gene; secY gene; thiG gene; trnA gene; tRNA-Ala; tRNA-Arg; tRNA-Asn; tRNA-Asp; tRNA-Cys; tRNA-fMet; tRNA-Gln; tRNA-Glu; tRNA-Gly; tRNA-His; tRNA-Ile; tRNA-Leu; tRNA-Lys; tRNA-Met; tRNA-Phe; tRNA-Pro; tRNA-Ser; tRNA-Thr; tRNA-Trp; tRNA-Tyr; tRNA-Val; trnC gene; trnD gene; trnE gene; trnF gene; trnfM gene; trnG gene; trnH gene; trnI gene; trnK gene; trnL gene; trnM gene; trnN gene; trnP gene; trnQ gene; trnR gene; trnS gene; trnT gene; trnV gene; trnW gene; trnY gene; trsE gene; tufA gene; ycf12 gene; ycf16 gene; ycf24 gene; ycf25 gene; ycf3 gene; ycf30 gene; ycf31 gene; ycf32 gene; ycf33 gene; ycf35 gene; ycf39 gene; ycf4 gene; ycf40 gene; ycf41 gene; ycf42 gene; ycf43 gene; ycf44 gene; ycf45 gene; ycf46 gene; ycf47 gene; ycf5 gene; ycf6 gene; ycf7 gene; ycf9 gene.

SOURCE chloroplast *Odontella sinensis*  
ORGANISM *Odontella sinensis*  
Eukaryota; stramenopiles; Bacillariophyta; Coscinodiscophyceae; Biddulphiophycidae; Eupodiscales; Eupodiscaceae; *Odontella*.

REFERENCE 1  
AUTHORS Kowallik, K.V., Stoebe, B., Schaffran, I., Kroth-Pancic, P. and Freier, U.  
TITLE The Chloroplast Genome of a chlorophyll a+c- containing Alga, *Odontella sinensis*  
JOURNAL Plant Mol. Biol. Rep. 13, 336-342 (1995)

REFERENCE 2 (bases 1 to 119704)  
AUTHORS Kowallik, K.V.  
TITLE Direct Submission  
JOURNAL Submitted (10-NOV-1995) K.V. Kowallik, Heinrich-Heine Universitaet Duesseldorf, Universitaetsstr. 1 Geb.26 13/02/46, D- 40225, Duesseldorf, FRG

FEATURES  
source Location/Qualifiers  
1..119704  
/organism="Odontella sinensis"  
/organelle="plastid:chloroplast"  
/db\_xref="taxon:2839"

gene 69086..70546  
/gene="ycf24"

CDS 69086..70546  
/gene="ycf24"  
/codon\_start=1  
/transl\_table=11  
/product="ORF486"  
/protein\_id="CAA91687.1"  
/db\_xref="GI:1185204"  
/db\_xref="SWISS-PROT:P49530"  
/translation="MTNKSNIKILNTNITKLVNQPYKYGFSTVIEKDIEKGLNEDVIC  
LISKKNNEPKFLLEFRLKAFKKWKEMKCPDWAQIKFSEIDYQDIYYSA PKVKKLNS  
LDEVDPPELLKTFEKLGISL TEQKRLANVAIDAVFDSVSIATTFKEELAECGVIFSSIS  
EAIQEYPELIEKYLGSVVPIDNYFSALNSAVFTDGSFCYIPKDTICPLELSTYFRIN  
DQKSGQFERTLIVAEKNSQVSYLEGCTAPQYDSNQLHAAVVELVALENADIKYSTVQN  
WYAGNNYEGGGIYNFVTKRGLCAGSNSKISWTQVETGSNITWKYPSCLLVGDKAKGEF  
YSVALTNNYQQADTGSKMIHVGNTRSRIVSKGISAGNSKN TYRGLVNI SNKAIGARN  
YSQCDSLLIGNLSNANTFPFISVQNPTAKIEHEASTSKIGEEQIFYFLQRGIPIEKGV  
ELMISGFCQEVFTELPLEFAAEADRLTLTLKLEGSVG"

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

17:: AJ132267. Skeletonema costa...[gi:4210403]

### Links

LOCUS SC0132267 542 bp DNA linear PLN 29-MAR-2001  
 DEFINITION Skeletonema costatum chromoplast ycf24 gene, partial.  
 ACCESSION AJ132267  
 VERSION AJ132267.1 GI:4210403  
 KEYWORDS ycf24 gene.  
 SOURCE chromoplast Skeletonema costatum  
 ORGANISM Skeletonema costatum  
 Eukaryota; stramenopiles; Bacillariophyta; Coscinodiscophyceae;  
 Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae;  
 Skeletonema.  
 REFERENCE 1  
 AUTHORS Tada,N., Otsuka,S., Oyaizu,H. and Matsumoto,S.  
 TITLE Plastid DNA sequences of Skeletonema costatum NIES 323  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 542)  
 AUTHORS Otsuka,S.  
 TITLE Direct Submission  
 JOURNAL Submitted (26-JAN-1999) Otsuka S., Applied Biological Chemistry,  
 The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, JAPAN  
 FEATURES Location/Qualifiers  
 source 1..542  
 /organism="Skeletonema costatum"  
 /organelle="plastid:chromoplast"  
 /strain="NIES 323"  
 /db\_xref="taxon:2843"  
 ...  
 gene 1..542  
 /gene="ycf24"  
 CDS <1..>542  
 /gene="ycf24"  
 /codon\_start=2  
 /transl\_table=11  
 /product="Ycf24 protein"  
 /protein\_id="CAA10636.1"  
 /db\_xref="GI:4210404"  
 /db\_xref="SPTREMBL:O96815"  
 /translation="AVFTDGSFCYIPKDVICPLDLSTYFRINDQNSGQFERTLIIAEE  
 NSKVSYLEGCTAPQYDNNQLHAAIVELIALKNATIKYSTVQNWYSGDQKGQGGVYNFV  
 TKRGLCAGDFSKISWTQVETGSSITWKYPSCVLVGDSAQGEFYVALTNNYQQADTGT  
 KMIHIGRNTRSRIVSKGISA"  
 BASE COUNT 186 a 74 c 109 g 173 t  
 ORIGIN  
 1 tgcagttttt acagatggat ctttttggtta tataacaaaa gatggtatgt gtcctctaga  
 61 tttatcaact tattttagga taaatgatca aaactctggt caatttgagc ggacattgat  
 121 tatagcagaa gaaaatagta aagttagtta tttagaagga tgtacagctc ctcaatatga  
 181 taataatcaa ctgcatgccg caattgtaga attaattgct ttaaaaaatg caacaataaa  
 241 atattctaca gttcaaaatt ggtattcttg ggacaaaaa gggcaagggt gtgtgtataa  
 301 ctttgtaaca aaacgagggtc tgtgtgcagg tgatttttct aagatttcgt ggactcaagt  
 361 tgaaactggt tcttcaataa cgtggaaata tctagttgt gtactagtgt gtgatagtgc  
 421 acaaggagaa ttttattcag ttgctttaac aaataactat caacaagctg acactggtac  
 481 aaaaatgatt catataggaa gaaatactcg aagccgtata gtttctaaag ggattttctgc  
 541 ag

# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

18: AF022186. Cyanidium caldarii...[gi:6466296]

### Links

LOCUS AF022186 164921 bp DNA circular PLN 14-DEC-2000  
 DEFINITION Cyanidium caldarium strain RK1 chloroplast, complete genome.  
 ACCESSION AF022186 Z36235 Z70297  
 VERSION AF022186.2 GI:6466296  
 KEYWORDS .  
 SOURCE chloroplast Cyanidium caldarium  
 ORGANISM Cyanidium caldarium  
 Eukaryota; Rhodophyta; Bangiophyceae; Porphyridiales;  
 Porphyridiaceae; Cyanidium.  
 REFERENCE 1 (bases 130696 to 132364)  
 AUTHORS Vogel,H., Fischer,S. and Valentin,K.  
 TITLE A model for the evolution of the plastid sec apparatus inferred  
 from secY gene phylogeny  
 JOURNAL Plant Mol. Biol. 32 (4), 685-692 (1996)  
 MEDLINE 97134960  
 PUBMED 8980520  
 REFERENCE 2 (bases 1 to 164921)  
 AUTHORS Glockner,G., Rosenthal,A. and Valentin,K.  
 TITLE The structure and gene repertoire of an ancient red algal plastid  
 genome  
 JOURNAL J. Mol. Evol. 51 (4), 382-390 (2000)  
 MEDLINE 20496959  
 PUBMED 11040290  
 REFERENCE 3 (bases 46857 to 47851)  
 AUTHORS Valentin,K.  
 TITLE Direct Submission  
 JOURNAL Submitted (22-MAR-1996) Institute for Plant Physiology, Justus  
 Liebig University, Heinrich Buff Ring 58-62, Giessen 35392, Germany  
 REFERENCE 4 (bases 28701 to 75580)  
 AUTHORS Gloeckner,G., Rosenthal,A. and Valentin,K.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-SEP-1997) Department of Genome Analysis, IMB Jena,  
 Beutenbergstr.11, Jena 07745, Germany  
 REFERENCE 5 (bases 1 to 164921)  
 AUTHORS Gloeckner,G., Rosenthal,A. and Valentin,K.  
 TITLE Direct Submission  
 JOURNAL Submitted (18-NOV-1999) Genome Analysis, Institute for Molecular  
 Biotechnology, Beutenbergstrasse 11, Jena 07745, Germany  
 REFERENCE 6 (bases 130696 to 132364)  
 AUTHORS Vogel,H., Fischer,S. and Valentin,K.  
 TITLE Direct Submission  
 JOURNAL Submitted (18-NOV-1999) Institute for Plant Physiology, Justus  
 Liebig University, Heinrich Buff Ring 58-62, Giessen 35392, Germany  
 COMMENT On or before Nov 23, 1999 this sequence version replaced gi:529651,  
 gi:1240002, gi:2465730.  
 FEATURES Location/Qualifiers  
 source 1..164921  
 /organism="Cyanidium caldarium"  
 /organelle="plastid:chloroplast"  
 /strain="RK1"  
 /db\_xref="taxon:2771"  
 gene 114386..115837  
 /gene="ycf24"

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

mRNA	/note="similar to ycf24 in Porphyra purpurea" 114386..115837 /gene="ycf24" /product="unknown"
CDS	114386..115837 /gene="ycf24" /codon_start=1 /transl_table=11 /product="unknown" /protein_id="AAF12948.1" /db_xref="GI:6466366" /translation="MIDRKKSSNIQNILNKPYKYGFSTEIQSEEFPGINEEIIRLMS HKKQEPDFILKFRLKAYQIWKKMQAPDWGHLHHNEINFNDVLCYASPKLEQGKKAQT ISEEILATFEKLGVPKPNKQPKIAVDAVFDSISFGTTLQKELKEQGIIFCSISEAI KAYPNLIKLYGSIVPAGDNYFAALNSAVFTDGSFCYIPKNIRCPVDLSTYFRINNKE AGQFERTLIIADENSFVNYLEGCTAPQFDTNQLHAAVVELICFKNATINYSTVQNWYA GNNKGEGGVYNFVTKRGLCQGENSKI SWTQLETGSAITWKYPSCLLKGKRSTGEFFSV TLTNNAQEADTGKMLHFRQSKSLVISKGISGGVSKNTYRGLVKISGSAIYSDNRSQ CDSL LIGKSESNTYPNLHVHNSLSKVEHEAFVSRIGEEQIFYFQQRGINIEEALNMI VSGFCQDVCNKLPMFALEANKLLNIKLEGSIG"

## APPENDIX D

### Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

19: AF138960. Neospora caninum ...[gi:6492292]

#### Links

```

LOCUS      AF138960          3343 bp    DNA        linear    INV 01-DEC-1999
DEFINITION Neospora caninum ycf24 protein (ycf24) gene, partial cds; DNA
            dependent RNA polymerase beta subunit (rpoB) gene, complete cds;
            and DNA dependent RNA polymerase beta subunit' (rpoC1) gene,
            partial cds, plastid genes for plastid products.
ACCESSION  AF138960
VERSION    AF138960.1  GI:6492292
KEYWORDS   .
SOURCE     plastid Neospora caninum
ORGANISM   Neospora caninum
            Eukaryota; Alveolata; Apicomplexa; Coccidia; Eimeriida;
            Sarcocystidae; Neospora.
REFERENCE  1 (bases 1 to 3343)
AUTHORS    Lang-Unnasch,N. and Aiello,D.P.
TITLE      Sequence evidence for an altered genetic code in the Neospora
            caninum plastid
JOURNAL     Int. J. Parasitol. 29 (10), 1557-1562 (1999)
MEDLINE     20074141
PUBMED      10608442
REFERENCE  2 (bases 1 to 3343)
AUTHORS    Lang-Unnasch,N. and Aiello,D.P.
TITLE      Direct Submission
JOURNAL     Submitted (26-MAR-1999) Div. Geographic Medicine, Dept. Medicine,
            University of Alabama at Birmingham, 845 S. 19th Street,
            Birmingham, AL 35294-2170, USA
FEATURES   Location/Qualifiers
            source          1..3343
                                /organism="Neospora caninum"
                                /organelle="plastid"
                                /strain="Nc1"
                                /db_xref="taxon:29176"
                                /note="the apicoplast is a vestigial nonphotosynthetic
                                plastid
                                plastid: apicoplast"
            gene            <1..43
                                /gene="ycf24"
            CDS              <1..43
                                /gene="ycf24"
                                /note="Toxoplasma gondii Ycf24 homolog"
                                /codon_start=2
                                /product="ycf24 protein"
                                /protein_id="AAF14260.1 [appellants note: pllivaraqk lfy]"
                                /db_xref="GI:6492293"
                                /translation="PLLIVARAQKLIFY"
            gene            75..3227
                                /gene="rpoB"
            CDS              75..3227
                                /gene="rpoB"
                                /note="Toxoplasma gondii RpoB and Plasmodium falciparum
                                RpoB homolog"
                                /codon_start=1
                                /transl_except=(pos:222..224,aa:Trp)
                                /transl_except=(pos:1866..1868,aa:Trp)
                                /transl_except=(pos:2976..2978,aa:Trp)

```

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```

/product="DNA dependent RNA polymerase beta subunit"
/protein_id="AAF14261.1"
/db_xref="GI:6492294"
/translation="MAFKIKTFLSIKSHKIFYTDFYFFLKKKLIILIKNIFPEYFNFN
NKYKNWNLICLPDFLYFKVNNTHFLDNVQYINSLIKIFLPLKFQNLKTNQIFPKNLLI
FELPKYNSHNYCYLNLGKKIFISKYFTSNGIFHKKYLKRKYDIYYAKILLTNSNFFNI
VLDLKLKQIYLSIKNLKFNFIPLYLGINNKDILKYSRYKSKILKLLIFTALQTNL
INNKKFILKNLNYLKSIFKVTNLNKNYKHFIINKNKNLYNYGNFNKNNFLTIDFIFIL
DLDDLQSKKLCFKNIDHLDNKHINTIGNYFQHNFKFYLLKFIISIIPNLIKLLKPSLL
KVYNFKELLILNPLIQYLEQINSFSELTHKYKLNNYNSSLKGILNLREICLNQIGKLC
LIDTTEGINCGLIVNFAKHIRIYKGGIIQIYVSPVFNKTKTEFINFKTSLDQELYLIQ
FNNINLRKNKLFNENIKVVYNKNNFRIKFISNKSFLKFADLFSFTENLIPFIKYND
PARCLMGAKMQSQSVPLLNKKKSFVVTGYEKEIITKSDITIKALQEGIVLNASSLKIH
IKDLFNREIVYYLSKYKKSQNQTLIHQKPLVWNGERVFTNQLLTQHQDIIDSEFAIGN
NLLIYYGNFCGYDFEDAVIGSKRVLYQQLFSSLHMDIYEFNFGYNNENDIEFSTLEIP
KQSYIYKKSLSLGIIVKEGKILTGNILLTKIKVTKPNYTYKSIIFKLIYSIFGKTIRN
IKDNSLYIQTGKNRVSKEIELFLINTNSHYKTYNNSYLKCRIFICKQRFITVGDKLCG
RYGNKGILSYIAENADLPFLQNSFYPDIIIVGALGIPSRMNLGQLFEALVGKIGFSYNI
RILPSFTSSSNAYFNLYLKILINFLMFNNLKKGFNWLYNPNLPGKFLIRDGRGTGILK
SSVLCGVSRYSKLIHMIKDKLHFRTTGPYTEILQQPLKGGKKNLGGQRFGEIWEALEA
FGASYNLKEILNYKSDDCFARNNLKDYLLFRNSELQNSTITESFRVILKEFNGLILNL
ELFLITDDLEENYLNLTINY"
gene      3243..>3343
CDS       /gene="rpoC1"
          3243..>3343
          /gene="rpoC1"
          /note="Toxoplasma gondii RpoC1 homolog"
          /codon_start=1
          /transl_except=(pos:3318..3320,aa:Trp)
          /product="DNA dependent RNA polymerase beta subunit"
          /protein_id="AAF14262.1"
          /db_xref="GI:6492295"
          /translation="MKKIYIKNNTIGFRLSLASPNLIKWSLKYIKN"
BASE COUNT      1416 a      272 c      306 g      1349 t
ORIGIN
    1 tcctcttttta attgtagcta gaggacaaaa attattttat taattttttg aatttaattgt
   61 aatttatttaa aattatggct tttaaaataa aaactttttt gtcaattaaa tcacataaaa
  121 tttttttatac tgatttttat ttttttttaa aaaaaaaatt aattatttta ataaaaaata
  181 tttttccaga atattttaat ttttaataata aatataaaaa ttgaaattta atttgcttc
  241 ctgacttctt atatttttaa gttaataata ctcatTTTTt agataatgtt caataataaa
  301 attctttaat aaaaatatTT ttaccattaa aatttcaaaa tttaaaaaca aatcaaatTT
  361 tttttaaaaa tttattaatt tttgagttac caaaatataa ttcacataat tattgttatt
  421 taaatggttt aaaaaaaatt tttatttcaa aatattttac ttcaaagggt atcttttttc
  481 ataaatatTT aaaaagaaaa tatgatattt attatgctaa aatattatta actaattcaa
  541 atttttttaa tattgtttta gatttaaaat tgaaacaaat ctatttatct attaaaaatt
  601 taaaatttaa ttttatttta tttttatatt atttaggaat taataataaa gatattttta
  661 aatattctag atataaaaaa tctaaaattt taaaattatt aatttttaca gctttacaaa
  721 ctaattttaat taataataaa aaatttatat taaaaaatct aaattattta aaatctattt
  781 ttaaagtaac aaatttaaat aaaaattata aacattttat aattaataaa aacaaattaa
  841 attataatta tggaaatttt aataaaaaata attttttaac tattgatttt atatttatat
  901 tagattttatt attagattta caatctaaaa aattatgttt taaaaatata gatcatttag
  961 ataataaaca tattaatata ataggtaatt attttcaaca taattttaaa ttctatttaa
 1021 aaaaatttat atctattata ccaaatttaa ttaaattaaa aaaattttct ttactaaaag
 1081 tatataattt taaagaatta ttaattctta acccattaat tcaatattta gaacaaatta
 1141 atagttttctc tgaacttacc cataaatata aattaaataa ttataattct tctttaaaag
 1201 gaatttttaa tctacgagaa atttgtttaa atcaaatagg taaactatgt ttaatagata
 1261 ctacagaagg aattaattgt ggtttaatag ttaactttgc taaacatatt agaatttata
 1321 aaaaaggcat aatacaaat tatgttagtc ctgtctttta aaataaaact aaagaattta
 1381 taaattttta aacatctcta gatcaagaat tatatttaaa tcaatttaaa aacattaatt

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## APPENDIX D

### Excerpts from 26 *yc24* gene product and gene sequences retrieved from NCBI database

```

1441 tacgtaaaaa taaattatct aatgaaaata tttaaagtagt atataataag aataatttta
1501 gaattaaatt tatttccaat aaaaaaagtt ttttattaaa atttgcagat ttattttcat
1561 ttacagaaaa tttaatacct tttattaaat ataatgaccc agcaagatgt ttaatgggag
1621 ctaagatgca aagtcaatca gttccattat taaataaaaa aaaatctttt gttgttactg
1681 ggatgaaaaa agaaataata actaaatctg atattactat taaagcttta caagaaggaa
1741 tagtttttaa tgcttcttct ttaaaaatac atattaaaga cctatttaat agagaaatag
1801 tttattatct atcaaaatat aaaaaaagta atcaaaacac attaatcat caaaaaccat
1861 tagtatgaaa tgggtgaaaga gtatttacta atcaattatt aacacaacat caagatatta
1921 ttgactcaga atttgcaata ggaaataatt tattgattta ttatggtaat ttttgtggat
1981 atgattttga agatgctgta attggtagta aaagagtttt atatcaacaa ttatttagtt
2041 ctcttcatat ggacatttat gaatttaatt ttgggtataa taatgaaaat gatattgaa
2101 ttagtacatt agaaattcct aaacaaagtt attatattaa aaaaagctta gattccttag
2161 gaattgttaa agaagggtga aaaattttta ctggtaatat tttattaca aaaataaaag
2221 ttacaaaacc taattataca tataaatcta tatttaaact tatatactct atttttggta
2281 aaacgattag aaatattaaa gataattctt tatatattca aacaggaaaa aatggtagag
2341 taagtaaaat tgaattatct ctaataaata caaattctca ttataaaact tataataaca
2401 gttattttaa atgtagaatt tttatatgta aacaaagatt ttttaactgt ggagataaat
2461 tatgtggaag atatggaaat aaagggattt tatcgtatat agcagaaaat gctgatttac
2521 cttttttaca aaattctttt tatccagata ttattgttgg tgcttttagt attccatcac
2581 gtatgaattt aggacaatta tttgaagctt tagttgggaa aataggtttt tcttataata
2641 ttagaatttt accttcattc acttcttctt ctaacgcgta ttttaattat ttaaaaattt
2701 taatatataa ttttttaatg ttcaataatc ttaaaaaagg ttttaattgg ctttataact
2761 ttaatttgcc aggaaaattt cttattagag atggaagaac tgggtataaaa ttaaaatctt
2821 cagttctttg tggagtttct agatattcaa aattaattca tatgattaag gataaacttc
2881 attttagaac aacaggacct tatactgaaa ttttacaaca acctttaaaa ggtaaaaaaa
2941 atttaggtgg tcaacgattt ggagaaatgg aaatttgagc tctagaagct tttggtgctt
3001 catataattt aaaagaaatt ttaaattata aatctgatga ttgtttcgca cgtaataatc
3061 tgaaagatta tttattatct agaaatagtg aattacaaaa ttcaactata actgaatctt
3121 ttcgtgttat tttaaaagaa tttaatggat taatttttaa tttagaatta tttttaataa
3181 cggacgattt agaagaaaat tatcttaatt taactattaa ttattaataa ttaaaattta
3241 tcatgaaaaa aatttatata aaaaacaata caataggttt tgcactatca ttagcttctc
3301 caaatttaat aattaaatga tctttaaat atataaaaaa ttt

```



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## Excerpts from 26 yc/24 gene product and gene sequences retrieved from NCBI database

20: U87145. Toxoplasma gondii... [gi:5231237]

### Links

LOCUS U87145 34996 bp DNA circular INV 29-JUN-1999  
 DEFINITION Toxoplasma gondii chloroplast, complete genome.  
 ACCESSION U87145  
 VERSION U87145.2 GI:5231237  
 KEYWORDS .  
 SOURCE chloroplast Toxoplasma gondii  
 ORGANISM Toxoplasma gondii  
 Eukaryota; Alveolata; Apicomplexa; Coccidia; Eimeriida;  
 Sarcocystidae; Toxoplasma.  
 REFERENCE 1 (bases 4337 to 4925)  
 AUTHORS Beckers,C.J., Roos,D.S., Donald,R.G., Luft,B.J., Schwab,J.C.,  
 Cao,Y. and Joiner,K.A.  
 TITLE Inhibition of cytoplasmic and organellar protein synthesis in  
 Toxoplasma gondii. Implications for the target of macrolide  
 antibiotics  
 JOURNAL Journal of Clin. Invest. 95, 367-376 (1995)  
 REFERENCE 2 (bases 13791 to 14996)  
 AUTHORS Kohler,S., Delwiche,C.F., Denny,P.W., Tilney,L.G., Webster,P.,  
 Wilson,R.J., Palmer,J.D. and Roos,D.S.  
 TITLE A plastid of probable green algal origin in Apicomplexan parasites  
 JOURNAL Science 275 (5305), 1485-1489 (1997)  
 MEDLINE 97197911  
 PUBMED 9045615  
 REFERENCE 3 (bases 13218 to 15870; 28669 to 34697)  
 AUTHORS Denny,P., Preiser,P., Williamson,D. and Wilson,I.  
 TITLE Evidence for a single origin of the 35 kb plastid DNA in  
 Apicomplexans  
 JOURNAL Protist 149, 51-59 (1998)  
 REFERENCE 4 (bases 1 to 34996)  
 AUTHORS Kissinger,J.C., Donald,R.G., Moulton,A.L., Gutell,R., Aiello,D.P.,  
 Lang-Unnasch,N. and Roos,D.S.  
 TITLE Mapping, cloning, and complete sequence annotation of the 35-kb  
 plastid genome of Toxoplasma gondii  
 JOURNAL Unpublished  
 REFERENCE 5 (bases 1 to 34996)  
 AUTHORS Kissinger,J.C., Donald,R.G., Moulton,A.L., Aiello,D.P.,  
 Lang-Unnasch,N. and Roos,D.S.  
 TITLE Direct Submission  
 JOURNAL Submitted (16-JAN-1997) Biology, University of Pennsylvania, 415 S.  
 University Ave, Philadelphia, PA 19104, USA  
 REFERENCE 6 (bases 1 to 34996)  
 AUTHORS Kissinger,J.C., Donald,R.G., Moulton,A.L., Aiello,D.P.,  
 Lang-Unnasch,N. and Roos,D.S.  
 TITLE Direct Submission  
 JOURNAL Submitted (28-JUN-1999) Biology, University of Pennsylvania, 415 S.  
 University Ave, Philadelphia, PA 19104, USA  
 REMARK Sequence update by submitter  
 COMMENT On Jun 28, 1999 this sequence version replaced gi:1870709.  
 This updated entry provides annotation (and corrected sequence  
 information) for the entire 35kb apicoplast genome of Toxoplasma  
 gondii, based on extensive resequencing from independent molecular  
 clones and a comprehensive reanalysis of the entire sequence.  
 Unlike the apicoplast genome of Plasmodium falciparum (Genbank  
 accession numbers X95275 & X95276) the T. gondii apicoplast genome

## APPENDIX D

### Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

exhibits non-standard codon usage: 33 in-frame UGA codons interrupt 17 of the 28 predicted coding regions, and are presumed to encode tryptophan. In addition, two genes (rps8 and rpoC2) contain in-frame UAA or UAG stop codons which are currently being investigated further. Note that the identification of many genes is hypothetical, based in part on the known organization of ribosomal protein superoperons in other taxa.

FEATURES	Location/Qualifiers
source	1..34996 /organism="Toxoplasma gondii" /organelle="plastid:chloroplast" /db_xref="taxon:5811"
..	
CDS	complement(28289..29686) /note="in frame UGA codon predicted to encode tryptophan; similar to Plasmodium falciparum plastid genome ORF470" /codon_start=1 /transl_except=(pos:complement(28916..28918),aa:Trp) /product="ycf24 homolog" /protein_id="AAD41153.1" /db_xref="GI:5231259" /translation="MKLYKYLYNKYNNNTDLFNTVRLIGGLNINMVNKLIFKQDNFIF LYIFRLNALSILNKFKQPDWCFYELPEFAFDDISYYSIPLNVYTNKNKYKSILSKLGL ELKFSENLILDVIFDSVLLNLTTFFLIKMGFLFFLSFFQSIIFYPYLIFSGLSIVSN TDNFFLTINSIIFNEGSFCFVMKDLNSNINLTTYFRTHSENFAQFERTLIVLSENSKL IYFEGCSAPMFLESQHLIAIVELFIKTKANLKYSTIQNWYRGNQLGEGGLYNFTTKRG FCMDKSFLNWIQIEIGSVITWKYPSTYLIGNKSFSNFFSLAMLSDYQVSDTGTKMLHI GKNTKSFILSKSLSFNFSFYTYRGLVTIFKTALNSYNYTECNSSLIGCNAFTATIPYT IINNFSAYINQEATISKLELDFLLHRGLNLKSTLMILIYGYCYNICSKISFELEL EVPLLIVARAQKLFY"

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

21:AF095904. *Toxoplasma gondii*...[gi:4336507]

#### Links

LOCUS	AF095904	3360 bp	DNA	linear	INV 04-MAR-1999
DEFINITION	<i>Toxoplasma gondii</i> ycf24 protein (ycf24) gene, partial cds; DNA dependent RNA polymerase beta subunit (rpoB) gene, complete cds; and DNA dependent RNA polymerase beta' subunit (rpoC1) gene, plastid genes encoding plastid proteins, partial cds.				
ACCESSION	AF095904				
VERSION	AF095904.1 GI:4336507				
KEYWORDS	.				
SOURCE	plastid <i>Toxoplasma gondii</i>				
ORGANISM	<i>Toxoplasma gondii</i> Eukaryota; Alveolata; Apicomplexa; Coccidia; Eimeriida; Sarcocystidae; <i>Toxoplasma</i> .				
REFERENCE	1 (bases 1 to 3360)				
AUTHORS	Aiello,D.P. and Lang-Unnasch,N.				
TITLE	Analysis of the rpoB gene product of <i>Toxoplasma gondii</i>				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 3360)				
AUTHORS	Aiello,D.P. and Lang-Unnasch,N.				
TITLE	Direct Submission				
JOURNAL	Submitted (30-SEP-1998) Geographic Medicine, University of Alabama at Birmingham, 845 South 19th Street, Birmingham, AL 35294-2170, USA				
FEATURES	Location/Qualifiers				
source	1..3360 /organism="Toxoplasma gondii" /organelle="plastid" /strain="RH" /db_xref="taxon:5811" /note="the apicoplast is a vestigial nonphotosynthetic plastid plastid: apicoplast"				
gene	<1..43				
CDS	/gene="ycf24" <1..43 /gene="ycf24" /note="ORF 470; similar to ORF 470 of Plasmodium falciparum apicoplast and Porphyra pupurea plastid" /codon_start=2 /product="ycf24 protein" /protein_id="AAD17841.1 [appellants note: pllivaraqk lfy]" /db_xref="GI:4336508" /translation="PLLIVARAQKLFY"				
gene	74..3229				
CDS	/gene="rpoB" 74..3229 /gene="rpoB" /EC_number="2.7.7.6" /codon_start=1 /transl_except=(pos:221..223,aa:Trp) /transl_except=(pos:1862..1864,aa:Trp) /transl_except=(pos:2750..2752,aa:Trp) /product="DNA dependent RNA polymerase beta subunit" /protein_id="AAD17842.1" /db_xref="GI:4336509"				

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```
/translation="MNFKIKTFFSIKSNKIFYTDFYFFLKKKLIILIKNIFPEYFNFN
NKYKNWNLIICLYDLLYFKVNNINFLDNVQYINSLIKIFLPLKFQNLKTNKVFFKNLLI
FELPKYNSYNCYLNLKKIFISKYFTSNGIFFNKYLKRKYNIYYAKLLLTNSNFFNI
ILDCLKQIYLSIKNLKFNFIILFYLLGIKNTDILKYSRYKNSKILKLLIFSALQTDL
VNNKKIILKNLNYLKSIFKLTIILKNYKNFIISKDKLNYNYGDFSKNNLLIIDFIFIL
DLLLLDQSKKLCFKTIDHLDNKHINTIGNYFQHNFKFYLLKKFISIIIPNLIKLQKFSLL
KVYNFKELLILNPLIQYLEQINSFSELMHXYKLNNYNSFSKGILNLREICNLQGLKLC
LIDTTEGINCGLVVSFAKHIRIYKKGIIQVYFSSIFKNKTKEFNLNFKTSLDQELYLIQ
FNNINLRKIKYLMNVRLVYNKNNFRIKFFSNKKSILLEFTDLFSFTENLIPFIKYNDP
ARCLMGAKMQSQSVPLLNKKKSFVITGYEKEIITKSDTTIKALQEGIVLNASSLKIHI
KDLFNREIVYYLSKYKKSQNQNTIIHQKPLVWNGERVFTNQLLTQHQDIIDSEFAIGNN
LLIYYGNFCGYDFEDAVIVSKRVLYQQLFSSSLHMDIYEFNFCYNNENDIEFSTLEIPK
QSYIYKKNLDSLGIKEGEKILTGSILLTKIKVAKPTYTYKSIKFLIYSIFGKTIRNI
KDNSLYIQTGKSGRVSKIELFLVNISRRHKYKTYNNSYLKCRIFICKQRFLTVGDKLC
GRYGNKGILSYIAENADLPFLQNSFYDPDIIVGALGIPSRMNLGQLFEALVGKISFSYN
IRILPSFTTSSNLYFNLYLKILYNFLMFNNFKKGFNWLYNPNLPGKFIIRDGRTGVKL
KSSVLCGVSRYSKLIHLIKDKLHFRRTTGPYTEILQQPLKGKKNLGGQRFGEIWALE
AFGASYNLKEILNYKSDDCFARNNLKEYLLFRNTELNSTITESFRVILKEFNGLIILN
LELFLITDDLEENYLNLTINY"
gene      3249..>3360
          /gene="rpoC1"
CDS       3249..>3360
          /gene="rpoC1"
          /codon_start=1
          /transl_except=(pos:3324..3326,aa:Trp)
          /product="DNA dependent RNA polymerase beta' subunit"
          /protein_id="AAD17843.1"
          /db_xref="GI:4336510"
          /translation="MKKIFIKNNTIGFRLSLASPNLIKWSLKYIKKFFYF"
BASE COUNT      1427 a      254 c      299 g      1380 t
ORIGIN
   1 tcctcttttta attgtagcaa gagcacaaaa attattttat taatttttta ttttaataaaa
  61 atttttttaaa attatgaatt ttaaaataaaa gacttttttt tcaattaaat caaataaaaat
121 tttttataact gattttttatt ttttttttaa aaaaaaatta attattttaa taaaaaatat
181 atttccagaa tatttttaatt ttaataataa atataaaaaat tgaaatttaa tttgtcttta
241 tgacttatta tatttttaaag ttaataatat taatttttta gataatgtac aatatataaa
301 ctctttaata aaaatttttt taccattaaa atttcaaaat ttaaaaacaa ataaagtttt
361 ttttaagaat ttattaattt ttgaattacc taaatataat tcatataatt attgttattt
421 aaatgggttta aaaaaaatat ttatttcaaa atattttact tcaaaggta tattttttta
481 taaatatttta aaaagaaaaa ataataatta ttatgctaaa ttattattaa caaatccaaa
541 tttttttaat attattttag atttaaaatt aaaacaaatt tatttatcta ttaaaaattt
601 aaaattttaat tttattttat ttttatatta ttttaggaatt aaaaatacag atatttttaa
661 atattctaga tataaaaatt ctaaaatttt aaaattatta attttttcag ctttacaac
721 tgatttagtt aataataaaa aaattatatt aaaaaattta aattatttaa aatctatttt
781 taaattaaca atttttaata aaaattataa aaattttata attagtaaag ataaattaaa
841 ttataattac ggagatttta gtaaaaaata tcttttaatt attgatttta tatttatatt
901 agatttatta ttagattttac aatctaaaaa attatgtttt aaaactatag atcatttaga
961 taataaacac attaatacaa taggtaatta ttttcaacat aatttttaaa tttattttaa
1021 aaaattttata tctattatac caaatttaat caaattacaa aaattttctt tattaaaagt
1081 atataatttt aaagaattat taattcttaa cccattaatt caatatttag aacaaattaa
1141 tagtttctca gaacttatgc ataaatataa attaaataat tataattctt tttcaaaagg
1201 aatttttaaa ttacgagaaa tttgttttaa tcaattaggt aaattatgtt taatagatac
1261 taccgaagga attaattgtg gtttagttgt tagttttgct aaacatatac gaatatataa
1321 aaaagggtata atacaagttt atttttagtc tatattttaa aataaaaacta aagaattttt
1381 aaatttttaa acatcttttag atcaagaatt atatttaatt caattttaata atattatttt
1441 acgtaaaata aaatatttaa tgaacgtagg attagtatat aataaaaaata attttagaat
1501 taaatttttt tctaataaaa aaagtatttt attagaattt acagatttat tttcatttac
1561 agaaaattta attcctttta ttaaatataa cgaccagca agatgcttaa tgggagctaa
1621 aatgcaaagt caatcagttc cattattaaa taaaaaaa tcttttgta ttactggata
```

**APPENDIX D**  
**Excerpts from 26 yc/24 gene product and gene sequences retrieved from NCBI database**

```
1681 tgaaaaagaa ataataacta aatcagatac tactattaaa gctctacaag aaggaatagt
1741 tttaaatgct tcttctttaa aaatacatat taaagattta tttaatagag agatagtata
1801 ttattttatca aaatataaaa aaagtaatca aaatacaata attcatcaaa aaccttttagt
1861 atgaaacggg gaaagagtat ttactaatca attactaaca caacatcaag atattataga
1921 ctcagaattt gcaataggaa ataatttatt aatttattat ggtaattttt gtggatatga
1981 ttttgaagat gctgtaattg ttagtaaaag agttttatat caacaattat tcagttctct
2041 tcatatggat ttttatgaat ttaatttttg ttataataat gaaaatgaca ttgaatttag
2101 tacattagaa atccctaaac aaagttatta tattaaaaaa aatttagatt ccttaggtat
2161 tattaaagaa ggtgaaaaaa ttttaactgg aagtatttta ctaacaaaaa taaaagtgc
2221 aaaacctact tatacttata aatctatatt taaacttata tattctattt ttggtaaaaa
2281 aattagaaat attaaagata attctttata tattcaaaca ggaaaaagcg gtagagtaag
2341 taaaattgaa ttatttttag taaatataag ttctcgtcat aaatataaaa cttataataa
2401 tagttattta aaatgtagaa tttttatatg taaacaaaga tttttaacag ttggagataa
2461 attatgtgga agatatggaa ataaaggaat tttatcttat atagcagaaa acgctgattt
2521 acctttttta caaaattctt tttatccaga tattattggt ggtgccttag gtattccatc
2581 tcgcatgaat ttaggacaat tatttgaagc tttagttggg aaaataagtt tttcttataa
2641 tattagaatt ttaccttcac ttactacttc ttctaattta ttttttaatt atctaaaaat
2701 tttaatatat aattttttta tgtttaataa ctttaaaaaa ggttttaatt gattatataa
2761 ttttaattta ccaggaaaat ttattattag agatggaaga actggtgtaa aattaaaatc
2821 ttcggttctt tgtggagttt ctaggtattc aaaattaatt catttgatta aagataaaact
2881 tcatttttaga actacaggac cttatactga aattttacaa caacctttta aaggtaaaaa
2941 aaatttagga ggtcaacgat ttggagaaat ggagatttgg gctttagaag cttttggagc
3001 ttcataataa ttaaaagaaa ttttaaatta taaatctgat gattgttttg cacgtaataa
3061 tcttaaagaa tattttattt ttagaaatac tgaactacaa aattcaacta ttactgaatc
3121 ttttcgcggt atttttaaaag aatttaattg attaatttta aatttagaat tttttttaat
3181 aacagacgat ttagaagaaa attatcttaa ttttaactatt aactattaat aaataattaa
3241 aattttattt gaaaaaaatc tttataaaaa ataatacaat aggtttttaga ttatcattag
3301 cttctoctaa ttttaataatt aaatgatctt taaaatatat aaaaaaattt ttttatttta
```

# APPENDIX D

## Excerpts from 26 yc/24 gene product and gene sequences retrieved from NCBI database

22: AF041468. *Guillardia theta* ...[gi:3602932]

### Links

LOCUS	AF041468	121524 bp	DNA	circular	PLN 03-MAR-1999
DEFINITION	<i>Guillardia theta</i> complete plastid genome.				
ACCESSION	AF041468 X14171 X62349 X51511 X14504 X52158 X52912 X56806 M76547 X62348 Z21976 U81044 AF063017				
VERSION	AF041468.1 GI:3602932				
KEYWORDS	.				
SOURCE	chloroplast <i>Guillardia theta</i>				
ORGANISM	<i>Guillardia theta</i> Eukaryota; Cryptophyta; Cryptomonadaceae; <i>Guillardia</i> .				
REFERENCE	1 (bases 47701 to 48415)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	The small subunit of ribulose-1,5-bisphosphate carboxylase is plastid-encoded in the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	Plant Mol. Biol. 13 (1), 13-20 (1989)				
MEDLINE	93357429				
PUBMED	2562756				
REFERENCE	2 (bases 18535 to 19351)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	Sequence analysis of the plastid rDNA spacer region of the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	DNA Seq. 1 (1), 55-62 (1990)				
MEDLINE	92119320				
PUBMED	2132959				
REFERENCE	3 (bases 43739 to 44938)				
AUTHORS	Douglas,S.E. and Durnford,D.G.				
TITLE	Nucleotide sequence of the genes for ribosomal protein S4 and tRNA(Arg) from the chlorophyll c-containing alga <i>Cryptomonas phi</i>				
JOURNAL	Nucleic Acids Res. 18 (7), 1903 (1990)				
MEDLINE	90245597				
PUBMED	2336372				
REFERENCE	4 (bases 34539 to 35380)				
AUTHORS	Reith,M. and Douglas,S.				
TITLE	Localization of beta-phycoerythrin to the thylakoid lumen of <i>Cryptomonas phi</i> does not involve a signal peptide				
JOURNAL	Plant Mol. Biol. 15 (4), 585-592 (1990)				
MEDLINE	91338697				
PUBMED	2102376				
REFERENCE	5 (bases 45872 to 47981)				
AUTHORS	Douglas,S.E., Durnford,D.G. and Morden,C.W.				
TITLE	Nucleotide sequence of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase from the chlorophyll c-containing Alga <i>Cryptomonas F</i> : evidence supporting the polyphyletic origin of plastids				
JOURNAL	J. Phycol. 26, 500-508 (1990)				
REFERENCE	6 (bases 110917 to 113854)				
AUTHORS	Douglas,S.E.				
TITLE	Unusual organization of a ribosomal protein operon in the plastid genome of <i>Cryptomonas phi</i> : evolutionary considerations				
JOURNAL	Curr. Genet. 19 (4), 289-294 (1991)				
MEDLINE	91330343				
PUBMED	1868578				
REFERENCE	7 (bases 40675 to 42376)				
AUTHORS	Douglas,S.E. and Turner,S.				

## APPENDIX D

### Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

TITLE	Molecular evidence for the origin of plastids from a cyanobacterium-like ancestor
JOURNAL	J. Mol. Evol. 33 (3), 267-273 (1991)
MEDLINE	92099311
PUBMED	1757997
REFERENCE	8 (bases 96129 to 98906)
AUTHORS	Wang,S.L. and Liu,X.Q.
TITLE	The plastid genome of <i>Cryptomonas phi</i> encodes an hsp70-like protein, a histone-like protein, and an acyl carrier protein
JOURNAL	Proc. Natl. Acad. Sci. U.S.A. 88 (23), 10783-10787 (1991)
MEDLINE	92073372
PUBMED	1961745
REFERENCE	9 (bases 106789 to 108216)
AUTHORS	Douglas,S.E.
TITLE	A secY homologue is found in the plastid genome of <i>Cryptomonas phi</i>
JOURNAL	FEBS Lett. 298 (1), 93-96 (1992)
MEDLINE	92183838
PUBMED	1544427
REFERENCE	10 (bases 42198 to 44153)
AUTHORS	Douglas,S.E. and Reith,M.E.
TITLE	A bchI homolog, encoding a subunit of Mg chelatase, is located on the plastid genomes of red and cryptomonad algae
JOURNAL	J. Mar. Biotechnol. 1, 135-141 (1993)
REFERENCE	11 (bases 82327 to 84479)
AUTHORS	Douglas,S.E. and Murphy,C.A.
TITLE	Structural, transcriptional and phylogenetic analyses of the atpB gene cluster from the plastid of <i>Cryptomonas F</i> (Cryptophyceae)
JOURNAL	J. Phycol. 30, 329-340 (1994)
REFERENCE	12 (bases 98901 to 114602)
AUTHORS	Wang,S.L., Liu,X.Q. and Douglas,S.E.
TITLE	The large ribosomal protein gene cluster of a cryptomonad plastid: gene organization, sequence and evolutionary implications
JOURNAL	Biochem. Mol. Biol. Int. 41 (5), 1035-1044 (1997)
MEDLINE	97283757
PUBMED	9137835
REFERENCE	13 (bases 61067 to 68605)
AUTHORS	Leitsch,C.E.W., Kowallik,K.V. and Douglas,S.E.
TITLE	The atpA gene cluster of a cryptomonad, <i>Guillardia theta</i> : A piece in the puzzle of chloroplast genome development
JOURNAL	J. Phycol. (1998) In press
REFERENCE	14 (bases 1 to 121524)
AUTHORS	Douglas,S.E. and Penny,S.L.
TITLE	The plastid genome of the cryptophyte alga, <i>Guillardia theta</i> : complete sequence and conserved syntenic groups confirm its common ancestry with red algae
JOURNAL	J. Mol. Evol. 48 (2), 236-244 (1999)
MEDLINE	99128221
PUBMED	9929392
REFERENCE	15 (bases 1 to 121524)
AUTHORS	Douglas,S.E.
TITLE	Direct Submission
JOURNAL	Submitted (08-JAN-1998) Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada
COMMENT	On or before Sep 15, 1998 this sequence version replaced gi:11396, gi:11297, gi:18103, gi:18281, gi:11383, gi:11407, gi:12539, gi:336730, gi:11300, gi:398949, gi:2661180.
FEATURES	Location/Qualifiers
source	1..121524

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

```
/organism="Guillardia theta"  
/organelle="plastid:chloroplast"  
/db_xref="taxon:55529"  
/note="plastid"
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...

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CDS       61067..62518  
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          /protein_id="AAC35664.1"  
          /db_xref="GI:3603003"  
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          KKKCEPSYMLDFRLKSYILWKKMSLPEWACLTYLNINYQDIVYYSAPKNSTKLDLED  
          VDKKILETFDKLGIPLNEQKKLANVAVD AIFDSVSVGTTFKQELSNVGVLCPLSEAT  
          NKYSTLVEKYLGSVVP IGDNYFAALNSAVFSEGSFCYI PPNVKCPLELSTYFRINNEN  
          SGQFERTLI IADFNSYVSYLEGCTAPMYDKNQLHAAVVELIALENAEIRYSTVQNWYS  
          GDTNGKGGIYNFVTKRGLCAGKSSKISWTQVETGSAITWKYPSCILVGEDSVGEFYVS  
          ALTNNYQQADTGTKMIHVGRGSKSRIISKGISAGYSKNTYRGQVKININALGSINNSQ  
          CDSMLIGPYSQANTYPYIQVSNAMSRVEHEASTSKIEEEQLFYFLQRGISVEQAISLL  
          ISGFCRDV FVKLPMEFAVEADKLLSVKLEGTVG"
```



# APPENDIX D

## Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

23:U38804. Porphyra purpurea... [gi:1276652]

### Links

LOCUS PPU38804 191028 bp DNA circular PLN 27-MAR-1998  
 DEFINITION Porphyra purpurea chloroplast, complete genome.  
 ACCESSION U38804  
 VERSION U38804.1 GI:1276652  
 KEYWORDS .  
 SOURCE chloroplast Porphyra purpurea  
 ORGANISM Porphyra purpurea  
 Eukaryota; Rhodophyta; Bangiophyceae; Bangiales; Bangiaceae;  
 Porphyra.  
 REFERENCE 1 (bases 1 to 191028)  
 AUTHORS Reith,M.E. and Munholland,J.  
 TITLE Complete nucleotide sequence of the Porphyra purpurea chloroplast  
 genome  
 JOURNAL Plant Mol. Biol. Rep. 13 (4), 333-335 (1995)  
 REFERENCE 2 (bases 1 to 191028)  
 AUTHORS Reith,M.E.  
 TITLE Direct Submission  
 JOURNAL Submitted (17-OCT-1995) Michael E. Reith, Marine Biology Section,  
 NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax,  
 Nova Scotia B3H 3Z1, Canada  
 FEATURES  
 source Location/Qualifiers  
 1..191028  
 /organism="Porphyra purpurea"  
 /organelle="plastid:chloroplast"  
 /strain="Avonport"  
 /db\_xref="taxon:2787"  
 ...  
 gene 40948..42411  
 /gene="ycf24"  
 CDS 40948..42411  
 /gene="ycf24"  
 /note="hypothetical chloroplast ORF 24."  
 /codon\_start=1  
 /protein\_id="AAC08126.1"  
 /db\_xref="GI:1276706"  
 /translation="MVNTQNQISQTSDDLIVNQPYKYGFTTSVESEQFPRGISREVV  
 KLISKKKNEPEYLLNFRLLKAYEKWTKMKNPKWAHLKHPNIDFNSIIYYAVPKLKKELN  
 SLDEVDP EILDTFNKLGLISLNEQKRLSNVAVDAVFDSVSIATTFKKELAEAGVIFCSI  
 SEAIRNYPDLIQKYLGTVPVPSGDNYFAALNSAVFSDGSFCYIPPDVTVCPLSTYFRI  
 NNEESGQFERTLIVADRGSKVSYLEGCTAPQYDTNQLHAAIVELIALDDAEIKYSTVQ  
 NNYAGNKDGGGIYNFVTKRGLCSGKNSKISWTQVETGSAITWKYPGCILAGDNSQGE  
 FYSVALTNNYQEADTGTMIHIGNNTKSKIIISKGISAGSKNSYRGLVKIGPQSFNSR  
 NYSQCDSLLIGQSSQANTFPYIQVNPTAKVEHEASTSKISEDQIFYFLQRGINLEES  
 VSLMISGFCKDVFNELPMEFAVEADRLLSLKLEGTVG"

**APPENDIX D**  
**Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database**

24:D90812. E.coli genomic DN...[gi:1742768]

Links

LOCUS D90812 13823 bp DNA linear BCT 29-MAY-1997  
DEFINITION E.coli genomic DNA, Kohara clone #321(38.1-38.4 min.).  
ACCESSION D90812 AB001340  
VERSION D90812.1 GI:1742768  
KEYWORDS Complete and shotgun sequencing; YCF24; ydiD.  
SOURCE Escherichia coli  
ORGANISM Escherichia coli  
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;  
Enterobacteriaceae; Escherichia.  
REFERENCE 1 (sites)  
AUTHORS Aiba,H., Baba,T., Fujita,K., Hayashi,K., Honjo,A., Horiuchi,T.,  
Ikemoto,K., Inada,T., Isono,K., Isono,S., Itoh,T., Kanai,K.,  
Kasai,H., Kashimoto,K., Kim,S., Kimura,S., Kitagawa,M.,  
Kitakawa,M., Makino,K., Masuda,S., Miki,T., Mizobuchi,K., Mori,H.,  
Motomura,K., Nakamura,Y., Nashimoto,H., Nishio,Y., Oshima,T.,  
Saito,N., Sampei,G., Seki,Y., Tagami,H., Takemoto,K., Wada,C.,  
Yamamoto,Y. and Yano,M.  
TITLE The systematic sequencing of the Escherichia coli genome in Japan  
JOURNAL Unpublished  
REFERENCE 2 (bases 1 to 13823)  
AUTHORS Mori,H.  
TITLE Direct Submission  
JOURNAL Submitted (14-DEC-1996) Hirotada Mori, NARA Institute of Science  
and Technology, Res. & Edu. Center for Genetic Info.; 8916-5  
Takayama, Ikoma, Nara 630-01, Japan  
(E-mail:hmori@gtc.aist-nara.ac.jp, Tel:81-7437-2-5660,  
Fax:81-7437-2-5669)  
COMMENT Collaboration Information:  
Project:  
The Japan E.coli genome DNA sequencing project  
Group:  
The Japan E.coli genome DNA sequencing group  
Members: (1995.4 - 1996.3)  
Aiba,H., Baba,T., Fujita,K., Hayashi,K., Honjo,A.,  
Horiuchi,T., Ikemoto,K., Inada,T., Isono,K., Isono,S.,  
Itoh,T., Kanai,K., Kasai,H., Kashimoto,K., Kim,S.,  
Kimura,S., Kitagawa,M., Kitakawa,M., Makino,K.,  
Masuda,S., Miki,T., Mizobuchi,K., Mori,H., Motomura,K.,  
Nakamura,Y., Nashimoto,H., Nishio,Y., Oshima,T., Saito,N.,  
Sampei,G., Seki,Y., Tagami,H., Takemoto,K., Wada,C.,  
Yamamoto,Y. and Yano,M.  
Headed by:  
Name: Takashi Horiuchi  
Address: National Institute of Basic Biology, Okazaki, 444, Japan  
E-mail: kishori@nibb.ac.jp  
Information operator:  
Name: Hirotada Mori  
Address: NARA Institute of Science and Technology,  
Ikoma, 630-01, Japan  
E-mail: hmori@gtc.aist-nara.ac.jp  
URL:  
The Japan E. coli genome database  
http:bsw3.aist-nara.ac.jp.

# APPENDIX D

## Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

```

FEATURES                      Location/Qualifiers
    source                     1..13823
                               /organism="Escherichia coli"
                               /strain="K12"
                               /db_xref="taxon:562"
                               /map="38.1 min"
                               /clone="Kohara clone #321"
                               /clone_lib="Kohara lambda miniset library"
                               /note="Nucleotide position 1773491-1787313 from the
                               initiation site of ThrA (0 min.).~This clone is from
                               Kohara lambda miniset library"
...
    gene                       888..4416
                               /gene="YCF24"
    CDS                         888..2414
                               /gene="YCF24"
                               /note="ORF_ID:o320#13
                               similar to [SwissProt Accession Number P48260]"
                               /codon_start=1
                               /transl_table=11
                               /protein_id="BAA15460.1"
                               /db_xref="GI:1742770"
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                               DELAKGINEEVVRAISAKRNEPEWMLEFRLNAYRAWLEMEEPHWLKAHYDKLNYQDYS
                               YYSAPSCGNCDTCCASEPGAVQQTGANAFLSKEVEAAFEQLGVPVREGKEVAVDIAFD
                               SVSVATTYREKLAEQGIIFCSFGGEAIHDHPELVVRKYLGTVPVPGNDNFFAALNAAVASD
                               GTFIYVPGKVRCPMELSTYFRINAECTGQFERTILVADEDSYVSYIEGCSAPVRDSYQ
                               LHAADVVEVI IHKNAEVKYSTVQNWFPDNNNTGGILNFVTKRALCEGENSKMSWTQSET
                               GSAITWKYPSCILRGDNSIGEFYSVALTSGHQQADTGTKMIHIGKNTKSTIISKGISA
                               GHSQNSYRGLVKIMPTATNARNFTQCDSMLIGANCGAHTFPYVECRNNSAQLEHEATT
                               SRIGEDQLFYCLQRGISEEDAISMIVNGFCKDVFSELPLEFAVEAQKLLAISLEHSVG
                               "

```

# APPENDIX D

## Excerpts from 26 *ycf24* gene product and gene sequences retrieved from NCBI database

25:D90811. E.coli genomic DN...[gi:1742754]

### Links

LOCUS D90811 19521 bp DNA linear BCT 29-MAY-1997  
 DEFINITION E.coli genomic DNA, Kohara clone #320(37.9-38.3 min.).  
 ACCESSION D90811 AB001340  
 VERSION D90811.1 GI:1742754  
 KEYWORDS Complete and shotgun sequencing; YCF24; aroD; ydiB; ydiF.  
 SOURCE Escherichia coli  
 ORGANISM Escherichia coli  
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.  
 REFERENCE 1 (sites)  
 AUTHORS Aiba,H., Baba,T., Fujita,K., Hayashi,K., Inada,T., Isono,K., Itoh,T., Kasai,H., Kashimoto,K., Kimura,S., Kitakawa,M., Kitagawa,M., Makino,K., Miki,T., Mizobuchi,K., Mori,H., Mori,T., Motomura,K., Nakade,S., Nakamura,Y., Nashimoto,H., Nishio,Y., Oshima,T., Saito,N., Sampei,G., Seki,Y., Sivasundaram,S., Tagami,H., Takeda,J., Takemoto,K., Takeuchi,Y., Wada,C., Yamamoto,Y. and Horiuchi,T.  
 TITLE A 570-kb DNA sequence of the Escherichia coli K-12 genome corresponding to the 28.0-40.1 min region on the linkage map  
 JOURNAL DNA Res. 3 (6), 363-377 (1996)  
 MEDLINE 97251357  
 PUBMED 9097039  
 REFERENCE 2 (sites)  
 AUTHORS Aiba,H., Baba,T., Fujita,K., Hayashi,K., Honjo,A., Horiuchi,T., Ikemoto,K., Inada,T., Isono,K., Isono,S., Itoh,T., Kanai,K., Kasai,H., Kashimoto,K., Kim,S., Kimura,S., Kitagawa,M., Kitakawa,M., Makino,K., Masuda,S., Miki,T., Mizobuchi,K., Mori,H., Motomura,K., Nakamura,Y., Nashimoto,H., Nishio,Y., Oshima,T., Saito,N., Sampei,G., Seki,Y., Tagami,H., Takemoto,K., Wada,C., Yamamoto,Y. and Yano,M.  
 TITLE The systematic sequencing of the Escherichia coli genome in Japan  
 JOURNAL Unpublished  
 REFERENCE 3 (bases 1 to 19521)  
 AUTHORS Mori,H.  
 TITLE Direct Submission  
 JOURNAL Submitted (14-DEC-1996) Hirotada Mori, NARA Institute of Science and Technology, Res. & Edu. Center for Genetic Info.; 8916-5 Takayama, Ikoma, Nara 630-01, Japan (E-mail:hmori@gtc.aist-nara.ac.jp, Tel:81-7437-2-5660, Fax:81-7437-2-5669)  
 COMMENT Collaboration Information:  
 Project:  
 The Japan E.coli genome DNA sequencing project  
 Group:  
 The Japan E.coli genome DNA sequencing group  
 Members: (1995.4 - 1996.3)  
 Aiba,H., Baba,T., Fujita,K., Hayashi,K., Honjo,A., Horiuchi,T., Ikemoto,K., Inada,T., Isono,K., Isono,S., Itoh,T., Kanai,K., Kasai,H., Kashimoto,K., Kim,S., Kimura,S., Kitagawa,M., Kitakawa,M., Makino,K., Masuda,S., Miki,T., Mizobuchi,K., Mori,H., Motomura,K., Nakamura,Y., Nashimoto,H., Nishio,Y., Oshima,T., Saito,N., Sampei,G., Seki,Y., Tagami,H., Takemoto,K., Wada,C., Yamamoto,Y. and Yano,M.

**APPENDIX D**  
**Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database**

Headed by:

Name: Takashi Horiuchi  
Address: National Institute of Basic Biology, Okazaki, 444, Japan  
E-mail: kishori@nibb.ac.jp

Information operator:

Name: Hirotada Mori  
Address: NARA Institute of Science and Technology,  
Ikoma, 630-01, Japan  
E-mail: hmori@gtc.aist-nara.ac.jp

URL:

The Japan E. coli genome database  
<http://bsw3.aist-nara.ac.jp>.

```
FEATURES
    source                Location/Qualifiers
                        1..19521
                        /organism="Escherichia coli"
                        /strain="K12"
                        /db_xref="taxon:562"
                        /map="37.9 min"
                        /clone="Kohara clone #320"
                        /clone_lib="Kohara lambda miniset library"
                        /note="Nucleotide position 1760879-1780399 from the
                        initiation site of ThrA (0 min.).~This clone is from
                        Kohara lambda miniset library"

...

    gene                13500..17028
                        /gene="YCF24"
    CDS                13500..15026
                        /gene="YCF24"
                        /note="ORF_ID:o320#13
                        similar to [SwissProt Accession Number P48260]"
                        /codon_start=1
                        /transl_table=11
                        /protein_id="BAA15454.1"
                        /db_xref="GI:1742763"
                        /translation="MWLWRKLGWIGGTMSRNTATDDVKTWTGGPLNYKEGFFTQLAT
                        DELAKGINEEVVRAISAKRNEPEWMLEFRLNAYRAWLEMEEPHWLKAHYDKLNYQDYS
                        YYSAPSCGNCDDTCASEPGAVQQTGANAFLSKEVEAAFEQLGVPVREGKEVAVDAlFD
                        SVSVATTYREKLAEQGIIFCSFGEAIHDHPELV RKYLGTVVPGNDNFFAALNAAVASD
                        GTFIYVPKGVRCPELMSTYFRINAECTGQFERTILVADEDSYVSYIEGCSAPVRDSYQ
                        LHAADVVEV I IHKNAEVKYSTVQNWFP GDNNTGGILNFVTKRALCEGENSKMSWTQSET
                        GSAITWKYPSCILRGDNSIGEFYSVALTSGHQQADTGT KMIHIGKNTKSTIISKGIS A
                        GHSQNSYRGLVKIMPTATNARNFTQCDSMLIGANCGAHTFPYVECRNNSAQLEHEATT
                        SRIGEDQLFYCLQRGISEEDAISMIVNGFCKDVFSELPLEFAVEAQKLLAISLEH SVG
                        "
```

## APPENDIX D

### Excerpts from 26 ycf24 gene product and gene sequences retrieved from NCBI database

26:U30821. Cyanophora parado... [gi:1016083]

#### Links

```

LOCUS      CPU30821                135599 bp    DNA      circular PLN 13-NOV-1995
DEFINITION Cyanophora paradoxa cyanelle, complete genome.
ACCESSION  U30821
VERSION    U30821.1  GI:1016083
KEYWORDS   .
SOURCE     cyanelle Cyanophora paradoxa
  ORGANISM Cyanophora paradoxa
            Eukaryota; Glaucocystophyceae; Cyanophoraceae; Cyanophora.
REFERENCE  1  (bases 1 to 135599)
  AUTHORS  Stirewalt,V.L., Michalowski,C.B., Luffelhardt,W., Bohnert,H.J. and
            Bryant,D.A.
  TITLE    Nucleotide sequence of the cyanelle genome from Cyanophora paradoxa
  JOURNAL  Unpublished
REFERENCE  2  (bases 1 to 135599)
  AUTHORS  Bryant,D.A.
  TITLE    Direct Submission
  JOURNAL  Submitted (01-JUL-1995) Donald A. Bryant, Biochemistry and
            Molecular Biology, The Pennsylvania State University, S-234 Frear
            Bldg., University Park, PA 16802, USA

FEATURES             Location/Qualifiers
     source            1..135599
                       /organism="Cyanophora paradoxa"
                       /organelle="plastid:cyanelle"
                       /strain="Pringsheim LB555"
                       /db_xref="taxon:2762"
...

     gene              complement(67893..69353)
                       /gene="ycf24"
     CDS                complement(67893..69353)
                       /gene="ycf24"
                       /codon_start=1
                       /product="ABC transporter subunit"
                       /protein_id="AAA81250.1"
                       /db_xref="GI:1016163"
                       /translation="MVNTQSPKNSGLENLVNQPYKYGLPLIFEIETISKGLTEETIRL
ISEKKNEPQFMLEFRLQAYRKWLEMSNEPEWAHLNYPKINYQDMVYYSAPKQKKKLQS
LDEVDP TLLTEFEKLGIPLTEQKRLANVAVD AIFDSVSVATTFKEELAKEGVIFCPIS
EAVQKYPDLIKKYLGSVVSTSDNYFSCLNAAVFS DGSFCYIPKNVRCPLELSTYFRIN
NGESGQFERTLIVADEGSYVSYLEGCTAPQFD TNQLHAAVVELVALDNAEIKYSTVQN
WYAGDENGKGGIYNFVTKRGLCAGKNSKISWTQVETGSAITWKYPSCVLLGDNSIGEF
YSVALTNRYQQADTGTKMIHIGKNTRSRIISKGISAGHSQNSYRGLVKIGPKAVGARN
YSQCDSL LIGDNSQANTFPHLQIKNPTAKVEHEASTSKIGEEQIFYFLQRGINAE EAI
SLIISGFCREVFNNLPMEFALEADKLLGLKLEGSVG"

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**Appendix E**  
**66 FR 1099, January 5, 2001**  
**"Guidelines for Examination of Patent**  
**Application Under 35 U.S.C. 12, ¶1, "Written**  
**Description Requirement"**

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 01-322 Filed 1-4-01; 8:45 am]

BILLING CODE 3510-16-U

## DEPARTMENT OF COMMERCE

### United States Patent and Trademark Office

[Docket No. 991027288-0264-02]

RIN 0651-AB10

### Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

**SUMMARY:** These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the *Federal Register* at 64 FR 71427, Dec. 21, 1999, and in the *Official Gazette* at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

**DATES:** The Guidelines are effective as of January 5, 2001.

**FOR FURTHER INFORMATION CONTACT:** Stephen Walsh by telephone at (703) 305-9035, by facsimile at (703) 305-9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therkorn by telephone at (703) 305-8800, by facsimile at (703) 305-8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therkorn@uspto.gov."

**SUPPLEMENTARY INFORMATION:** As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

### Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications

Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the *Federal Register* at 64 FR 71427, Dec. 21, 1999, and in the *Official Gazette* at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

### Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site ([www.uspto.gov](http://www.uspto.gov)). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

### Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers v. Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,



formula, or definitive chemical or physical properties.").

*Response:* The suggestions have been adopted in part. The purpose of the written description analysis is to confirm that applicant had possession of what is claimed. The Guidelines have been modified to instruct the examiners to compare the scope of the invention claimed with the scope of what applicant has defined in the description of the invention. That is, the Guidelines instruct the examiner to look for consistency between a claim and what provides adequate factual support for the claim as judged by one of ordinary skill in the art from reading the -- corresponding written description.

(2) *Comment:* Two comments urge that *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997), is bad law and should not be followed by the USPTO because it conflicts with binding precedent, such as *Vas-Cath v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed. Cir. 1991). *Response:* The final Guidelines are based on the Office's current understanding of the law and are believed to be fully consistent with binding precedent of the U.S. Supreme Court and the U.S. Court of Appeals for the Federal Circuit. *Eli Lilly* is a precedential decision by the Court that has exclusive jurisdiction over appeals involving patent law. Accordingly, the USPTO must follow *Eli Lilly*. Furthermore, the USPTO does not view *Eli Lilly* as conflicting with *Vas-Cath*. *Vas-Cath* explains that the purpose of the written description requirement is to ensure that the applicant has conveyed to those of skill in the art that he or she was in possession of the claimed invention at the time of filing. *Vas-Cath*, 935 F.2d at 1563-64, 19 USPQ2d at 1117. *Eli Lilly* explains that a chemical compound's name does not necessarily convey a written description of the named chemical compound, particularly when a genus of compounds is claimed. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1405. The name, if it does no more than distinguish the claimed genus from all others by function, does not satisfy the written description requirement because "it does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. Thus, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to

others that applicants had possession of what they claimed.

(3) *Comment:* Several comments urged that the Guidelines do not recognize the inconsistency between the original claim doctrine and the written description requirement as set out in *Fiers* and *Eli Lilly*. On the other hand, another comment asserts that there is no strong presumption that an originally filed claim constitutes an adequate written description of the claimed subject matter. Several comments indicate that *in haec verba* support should be sufficient to comply with the written description requirement. Two comments urge that the concept of constructive reduction to practice upon filing of an application has been ignored. *Response:* As noted above, the USPTO does not find *Fiers* and *Eli Lilly* to be in conflict with binding precedent. An original claim may provide written description for itself, but it still must be an adequate written description which establishes that the inventor was in possession of the invention. The "original claim doctrine" is founded on cases which stand for the proposition that originally filed claims are part of the written description of an application as filed, and thus subject matter which is present only in originally filed claims need not find independent support in the specification. See, e.g., *In re Koller*, 613 F.2d 819, 824, 204 USPQ 702, 706 (CCPA 1980) (later added claims of similar scope and wording were adequately described by original claims); *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149, 149 (CCPA 1973) ("Under these circumstances, we consider the original claim in itself adequate 'written description' of the claimed invention. It was equally a 'written description' \* \* \* whether located among the original claims or in the descriptive part of the specification."). However, as noted in the preceding comment, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to others that applicants had possession of what they claimed. When the name of a novel chemical compound does not convey sufficient structural information about the compound to identify the compound, merely reciting the name is not enough to show that the inventor had possession of the compound at the time the name was written. The Guidelines indicate that there is a "strong presumption" that an adequate written description of the claimed invention is present when the application is filed, consistent with *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ

90, 97 (CCPA 1976) ("We are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). In most cases, the statement that "an originally filed claim is its own written description," is borne out because the claim language conveys to others of skill in the art that the applicant was "in possession" of what is claimed. The Guidelines emphasize that the burden of proof is on the examiner to establish that a description as filed is not adequate and require the examiner to introduce sufficient evidence or technical reasoning to shift the burden of going forward with contrary evidence to the applicant.

(4) *Comment:* One comment stated that the Guidelines change the substance of the written description requirement to require some level of enablement. The comment stated that the *Eli Lilly* case should not be followed because its change in the quality of the description required is in conflict with precedent. Another comment suggested that to comply with the written description requirement, the description must both (i) demonstrate possession of the claimed invention by the applicant; and (ii) put the public in possession of the claimed invention. *Response:* As noted in the comment above, the USPTO is bound by the Federal Circuit's decision in *Eli Lilly*. The Guidelines have been revised to clarify that an applicant must provide a description of the claimed invention which shows that applicant was in possession of the claimed invention. The suggestion to emphasize that the written description requirement must put the public in possession of the invention has not been adopted because it removes much of the distinction between the written description requirement and the enablement requirement. Although the two concepts are entwined, they are distinct and each is evaluated under separate legal criteria. The written description requirement, a question of fact, ensures that the inventor conveys to others that he or she had possession of the claimed invention; whereas, the enablement requirement, a question of law, ensures that the inventor conveys to others how to make and use the claimed invention.

(5) *Comment:* One comment suggested that the Guidelines should provide examples of situations in which the written description requirement was met but the enablement requirement was not, and vice versa. Another comment stated that examiners often use enablement language in making

written description rejections.

**Response:** The enablement and written description requirements are not coextensive and, therefore, situations will arise in which one requirement is met but the other is not. Federal Circuit case law demonstrates many circumstances where enablement or written description issues, but not both, were before the Court. These Guidelines are intended to clarify for the examining corps the criteria needed to satisfy the written description requirement. For examples applying these Guidelines to hypothetical fact situations, see the "Synopsis of Application of Written Description Guidelines" (examiner training materials available on-line at <http://www.uspto.gov/web/menu/written.pdf>). These examples, as well as the examination form paragraphs and instructions on their proper use, provide the appropriate language examiners should use in making written description rejections.

(6) **Comment:** One comment disagreed with the statement in an endnote that "the fact that a great deal more than just a process is necessary to render a product invention obvious means that a great deal more than just a process is necessary to provide written description for a product invention." The comment indicated that the statement is overly broad and inconsistent with the "strong presumption that an adequate written description of the claimed invention is present when the application is filed." As an extreme case, for example, for product-by-process claims, nothing else would be needed to provide the written description of the product. **Response:** The endnote has been clarified and is now more narrowly drawn. However, there is no *per se* rule that disclosure of a process is sufficient to adequately describe the products produced by the process. In fact, *Fiers v. Revel* and *Eli Lilly* involved special circumstances where the disclosure of a process of making and the function of the product alone did not provide an adequate written description for product claims. Even when a product is claimed in a product-by-process format, the adequacy of the written description of the process to support product claims must be evaluated on a case-by-case basis.

(7) **Comment:** Several comments urge that actual reduction to practice, as a method of satisfying the written description requirement by demonstrating possession, has been over-emphasized. **Response:** The Guidelines have been clarified to state that describing an actual reduction to practice is one of a number of ways to show possession of the invention.

Description of an actual reduction to practice offers an important "safe haven" that applies to all applications and is just one of several ways by which an applicant may demonstrate possession of the claimed invention. Actual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps, in such a way as to distinguish the composition with particularity from all others. Thus, the emphasis on actual reduction to practice is appropriate in those cases where the inventor cannot provide an adequate description of what the composition is, and a definition by function is insufficient to define a composition "because it is only an indication of what the [composition] does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ at 1406. See also *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991).

(8) **Comment:** One comment asserts that the citation to *Pfaff v. Wells Electronics, Inc.*, 525 U.S. 55, 48 USPQ2d 1641 (1998) is inappropriate and should be deleted because *Pfaff* is concerned with § 102(b) on-sale bar, not written description. Another comment suggested that the Guidelines should provide an explanation of how the "ready for patenting" concept of *Pfaff* should be used in determining compliance with the written description requirement. **Response:** The Guidelines state the general principle that actual reduction to practice is not required to show possession of, or to adequately describe, a claimed invention (although, as noted in the previous comment, an actual reduction to practice is crucial in relatively rare instances). An alternative is to show that the invention described was "ready for patenting" as set out in *Pfaff*. For example, a description of activities that demonstrates the invention was "ready for patenting" satisfies the written description requirement. As *Wertheim* indicates, "how the specification accomplishes this is not material." 541 F.2d at 262, 191 USPQ at 96.

(9) **Comment:** One comment stated that the written description of a claimed DNA should be required to include the complete sequence of the DNA and claims should be limited to the DNA sequence disclosed. **Response:** Describing the complete chemical structure, i.e., the DNA sequence, of a claimed DNA is one method of

satisfying the written description requirement, but it is not the only method. See *Eli Lilly*, 119 F.3d at 1566, 43 USPQ2d at 1404 ("An adequate written description of a DNA . . . requires a precise definition, such as by structure, formula, chemical name, or physical properties." (emphasis added, internal quote omitted)). Therefore, there is no basis for a *per se* rule requiring disclosure of complete DNA sequences or limiting DNA claims to only the sequence disclosed.

(10) **Comment:** One comment stated that it is difficult to envision how one could provide a description of sufficient identifying characteristics of the invention without physical possession of a species of the invention, and thus this manner of showing possession should be considered as a way to show actual reduction to practice. **Response:** This suggestion has not been adopted. The three ways of demonstrating possession as set forth in the Guidelines are merely exemplary and are not mutually exclusive. While there are some cases where a description of sufficient relevant identifying characteristics will evidence an actual reduction to practice, there are other cases where it will not. See, e.g., *Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1576, 227 USPQ 177, 180 (Fed. Cir. 1985) (disclosure taken with the knowledge of those skilled in the art may be sufficient support for claims).

(11) **Comment:** One comment stated that the Guidelines should be revised to indicate that the test of disclosure of sufficiently detailed drawings should be expanded to include structural claiming of chemical entities. **Response:** The suggestion has been adopted.

(12) **Comment:** One comment stated that the Guidelines should reflect that an inventor is in possession of the invention when the inventor demonstrably has at least a complete conception thereof, and that factors and attributes which provide proof of written description should include evidence typically provided to prove a complete conception. **Response:** The suggestion has not been adopted because the conception analysis typically involves documentary evidence in addition to the description of the invention in the application as filed. However, it is acknowledged that if evidence typically provided to prove a complete conception is present in the specification as filed, it would be sufficient to show possession. The Federal Circuit has stated "[t]he conception analysis necessarily turns on the inventor's ability to describe his invention with particularity. Until he can do so, he cannot prove possession

of the complete mental picture of the invention." *Burroughs Wellcome Co. v. Barr Labs., Inc.*, 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994). As further noted by the Federal Circuit, in order to prove conception, "a party must show possession of every feature recited in the count, and that every limitation of the count must have been known to the inventor at the time of the alleged conception." *Coleman v. Dines*, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed. Cir. 1985).

(13) *Comment*: One comment indicated that a "possession" test does not appear in Title 35 of the U.S. Code and is not clearly stated by the Federal Circuit. Therefore, it is recommended that patent examiners be directed to use existing judicial precedent to make rejections of claims unsupported by a statutory written description requirement. *Response*: While the Federal Circuit has not specifically laid out a "possession" test, the Court has clearly indicated that possession is a cornerstone of the written description inquiry. See, e.g., *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991); see also *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) ("[o]ne skilled in the art, reading the disclosure, must immediately discern the limitation at issue in the claims") (internal quote omitted). The possession test as set forth in the Guidelines is extrapolated from case law in a wide variety of technologies and is not intended to be limiting. Any rejections made by examiners will be made under 35 U.S.C. 112, ¶1, with supporting rationale. Final rejections are appealable if applicant disagrees and follows the required procedures to appeal.

(14) *Comment*: Two comments indicated that if the amino acid sequence for a polypeptide whose utility has been identified is described, then the question of possession of a class of nucleotides encoding that polypeptide can be addressed as a relatively routine matter using the understanding of the genetic code, and that the endnote addressing this issue should be revised. *Response*: The suggestion of these comments has been incorporated in the Guidelines and will be reflected in the training materials. However, based upon *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994), this does not mean that applicant was in possession of any particular species of the broad genus.

(15) *Comment*: One comment disagreed with an endnote which stated

that a laundry list disclosure of moieties does not constitute a written description of every species in a genus. Specifically, the comment indicates that if the existence of a functional genus is adequately described in the specification, a laundry list of the species within that genus must satisfy the written description requirement.

*Response*: The suggestion to revise the endnote will not be adopted. A lack of adequate written description problem arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosure. This was aptly demonstrated in *In re Bell* and *In re Baird* where possession of a large genus did not put a person of ordinary skill in the art in possession of any particular species. See also *Purdue Pharma*, 230 F.3d at 1328, 56 USPQ2d at 1487 (because the original specification did not disclose the later claimed concentration ratio was a part of the invention, the inventors cannot argue that they are merely narrowing a broad invention).

(16) *Comment*: One comment suggested that in the majority of cases, a single species will support a generic claim, and that the Guidelines should emphasize this point. *Response*: The suggestion has been adopted to a limited degree. The Guidelines now indicate that a single species may, in some instances, provide an adequate written description of a generic claim when the description of the species would evidence to one of ordinary skill in the art that the invention includes the genus. Note, however, *Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998), where the species in the parent application was held not to provide written description support for the genus in the child application.

(17) *Comment*: One comment asserted that the Guidelines should focus on the compliance of the claims, not the specification, with the written description requirement. *Response*: This suggestion will not be adopted. "The specification shall contain a written description of the invention." 35 U.S.C. 112. The claims are part of the specification. *Id.*, ¶ 2. If an adequate description is provided, it will suffice "whether located among the original claims or in the descriptive part of the specification." *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149 (CCPA 1973). The entire disclosure, including the specification, drawings, and claims, must be considered.

(18) *Comment*: One comment asserted that the Guidelines confuse "new matter." 35 U.S.C. 132, with the written description requirement, and that the

same standard for written description should be applied to both original claims and new or amended claims.

*Response*: The Guidelines indicate that for both original and amended claims, the inquiry is whether one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention at the time the application was filed.

(19) *Comment*: One comment suggested that the second paragraph of the section pertaining to determining what the claim as a whole covers should be deleted because it relates more to compliance with § 112, second paragraph, than with the written description requirement. *Response*: This suggestion will not be adopted. The claims must be construed and all issues as to the scope and meaning of the claim must be explored during the inquiry into whether the written description requirement has been met. The concept of treating the claim as a whole is applicable to all criteria for patentability.

(20) *Comment*: One comment suggested a different order for the general analysis for determining compliance with the written description requirement, starting with reading the claim, then the specification, and then determining whether the disclosure demonstrates possession by the applicant. *Response*: This suggestion will not be adopted. The claims must be construed as broadly as reasonable in light of the specification and the knowledge in the art. See *In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Then the disclosure must be evaluated to determine whether it adequately describes the claimed invention, i.e., whether it conveys to a person having ordinary skill in the art that the applicant had possession of what he or she now claims.

(21) *Comment*: Several comments suggested that the Guidelines are unclear with regard to how the examiner should treat the transitional phrase "consisting essentially of." The comments also suggested that the endnote that explains "consisting essentially of" does not make clear how the use of this intermediate transitional language affects the scope of the claim. Several comments stated that the USPTO does not have legal authority to treat claims reciting this language as open (equivalent to "comprising"). Another comment suggested that the phrase "clear indication in the specification" be replaced with "explicit or implicit indication." *Response*: The transitional phrase "consisting essentially of" excludes

ingredients that would 'materially affect the basic and novel characteristics' of the claimed composition." *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 1574, 224 USPQ 409, 412 (Fed. Cir. 1984). The basic and novel characteristics of the claimed invention are limited by the balance of the claim. *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 896 (CCPA 1963). However, during prosecution claims must be read broadly, consistent with the specification. *In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Thus, for purposes of searching for and applying prior art in a rejection under 35 U.S.C. 102 or 103, if the specification or the claims do not define the "basic and novel" properties of the claimed subject matter (or if such properties are in dispute), the broadest reasonable interpretation consistent with the specification is that the basic and novel characteristics are merely the presence of the recited limitations. See, e.g., *Janakirama-Rao*, 317 F.2d at 954, 137 USPQ at 895-96. This does not indicate that the intermediate transitional language is never given weight. Applicants may amend the claims to avoid the rejections or seek to establish that the specification provides definitions of terms in the claims that define the basic and novel characteristics of the claimed invention which distinguish the claimed invention from the prior art. When an applicant contends that additional steps or materials in the prior art are excluded by the recitation of 'consisting essentially of,' applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964). The language used in the Guidelines is consistent with *PPG Industries Inc. v. Guardian Industries Corp.*, 156 F.3d 1351, 1355, 48 USPQ2d 1351, 1355 (Fed. Cir. 1998) ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics.").

(22) *Comment*: One comment stated that the written description should "disclose the invention," including why the invention works and how it was developed. *Response*: This suggestion has not been adopted. An inventor does not need to know how or why the invention works in order to obtain a patent. *Newman v. Quigg*, 877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345

(Fed. Cir. 1989). To satisfy the enablement requirement of 35 U.S.C. 112, ¶ 1, an application must disclose the claimed invention in sufficient detail to enable a person of ordinary skill in the art to make and use the claimed invention. To satisfy the written description requirement of 35 U.S.C. 112, ¶ 1, the description must show that the applicant was in possession of the claimed invention at the time of filing. There is no statutory basis to require disclosure of why an invention works or how it was developed. "Patentability shall not be negated by the manner in which the invention was made." 35 U.S.C. 103(a).

(23) *Comment*: One comment recommended that the phrases "emerging and unpredictable technologies" and "unpredictable art" be replaced with the phrase—"inventions characterized by factors which are not reasonably predictable in terms of the ordinary skill in the art—". *Response*: The suggestion is adopted in part and the recommended phrase has been added as an alternative.

(24) *Comment*: One comment recommended that the phrase "conventional in the art" be replaced with—"part of the knowledge of one of ordinary skill in the art—". *Response*: The suggestion is adopted in part and the recommended phrase has been added as an alternative. The standard of "conventional in the art" is supported by case law holding that a patent specification "need not teach, and preferably omits, what is well known in the art." See *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1534, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). See also *Atmel Corp. v. Information Storage Devices, Inc.*, 198 F.3d 1374, 1382, 53 USPQ2d 1225, 1231 (Fed. Cir. 1999).

(25) *Comment*: One comment recommended that the Guidelines be amended to state that the appropriate skill level for determining possession of the claimed invention is that of a person of ordinary skill in the art. *Response*: The comment has not been adopted. The statutory language itself indicates that compliance with the requirements of 35 U.S.C. 112, ¶ 1, is judged from the standard of "any person skilled in the art." It is noted, however, that the phrases "one of skill in the art" and "one of ordinary skill in the art" appear to be synonymous. See, e.g., *Union Oil Co. v. Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ2d 1227, 1232 (Fed. Cir. 2000) ("The written description requirement does not require the applicant to describe exactly the subject

matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." Thus, § 112, ¶ 1, ensures that, as of the filing date, the inventor conveyed with reasonable clarity to those of skill in the art that he was in possession of the subject matter of the claims." (citations omitted, emphasis added)).

(26) *Comment*: One comment stated that an endnote misstates the relevant law in stating that, to show inherent written descriptive support for a claim limitation, the inherent disclosure must be such as would be recognized by a person of ordinary skill in the art. The comment recommended that the endnote be amended to delete the reference to recognition by persons of ordinary skill and to cite *Pingree v. Hull*, 518 F.2d 624, 186 USPQ 248 (CCPA 1975), rather than *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949 (Fed. Cir. 1999). *Response*: The comment has not been adopted. Federal Circuit precedent makes clear that an inherent disclosure must be recognized by those of ordinary skill in the art. See, e.g., *Hyatt v. Boone*, 146 F.3d 1348, 1354-55, 47 USPQ2d 1128, 1132 (Fed. Cir. 1998) ("[T]he purpose of the description requirement is 'to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter later claimed by him.' \* \* \* Thus, the written description must include all of the limitations of the interference count, or the applicant must show that any absent text is necessarily comprehended in the description provided and would have been so understood at the time the patent application was filed." (emphasis added)). See also *Reiffin v. Microsoft Corp.*, 214 F.3d 1342, 1346, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000) (The "application considered as a whole must convey to one of ordinary skill in the art, either explicitly or inherently, that [the inventor] invented the subject matter claimed \* \* \*." See \* \* \* *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (descriptive matter may be inherently present in a specification if one skilled in the art would necessarily recognize such a disclosure)).

(27) *Comment*: Several comments pointed out an inconsistency in the Federal Register Notice re: the Revised Interim Written Description Guidelines. The inconsistency concerned the treatment of claims directed to an isolated DNA comprising SEQ ID NO:1 wherein SEQ ID NO:1 is an expressed sequence tag. The comments contrasted paragraphs 34 and 35 of the Response to

Public Comments with the statement in the text of the Guidelines that a genus must be supported by a representative number of species (as analyzed in Example 7 of the training materials). *Response:* The USPTO acknowledges that there was an inconsistency. The Office notes that a claim reciting a nucleic acid comprising SEQ ID NO:1 may be subject to a rejection for lack of an adequate written description where particular identifiable species within the scope of the claim lack an adequate written description. The training materials as amended exemplify an appropriate analysis.

(28) *Comment:* One comment stated that the USPTO should respond to the issue of whether the U.S. is meeting its TRIPs obligations. This comment noted that the USPTO did not address an earlier comment regarding the "Interim Guidelines for the Examination of Patent Applications under the 35 U.S.C. 112, § 1, 'Written Description' Requirement." 63 FR 32,639, June 15, 1998, which questioned whether the written description requirement is truly different from the enablement requirement, and indicated that such a requirement may be contrary to the TRIPs provisions of the World Trade Organization (Article 27.1). Article 27.1 requires WTO Members to, *inter alia*, make patents available, with limited exceptions, for products and processes in all fields of technology so long as those products and processes are new, involve an inventive step, and are capable of industrial application. The comment further suggested a response. *Response:* TRIPs Article 27 does not address what must be included in a patent application to allow WTO Member officials to determine whether particular inventions meet the standards for patentability established in that Article. TRIPs Article 29, which is more relevant to this comment, states that Members "shall require" patent applicants to disclose their invention "in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art." If the written description is not clear and complete, the applicant may not have been in possession of the invention. This may support both written description and enablement standards. In addition, Article 29 expressly authorizes Members to require patent applicants to disclose the best method the inventor knows at the time of filing an application for carrying out the invention.

(29) *Comment:* Two comments commended the USPTO for eliminating the Biotechnology Specific Examples in the Revised Interim Written Description

Guidelines and providing separate training materials. One comment indicated a need to reconfirm the examples set forth in the Interim Written Description Guidelines published in 1998. *Response:* The current training materials reflect the manner in which the USPTO interprets the Written Description Guidelines.

(30) *Comment:* Several comments addressed specific concerns about the examiner training materials. *Response:* The comments received with respect to the training materials will be taken under advisement as the Office revises the training materials in view of the revisions to the Guidelines. The specific comments will not be addressed herein as they do not impact the language of the Guidelines.

#### **Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, § 1, "Written Description" Requirement**

These "Written Description Guidelines" are intended to assist Office personnel in the examination of patent applications for compliance with the written description requirement of 35 U.S.C. 112, § 1. This revision is based on the Office's current understanding of the law and public comments received in response to the USPTO's previous request for public comments on its Revised Interim Written Description Guidelines and is believed to be fully consistent with binding precedent of the U.S. Supreme Court, as well as the U.S. Court of Appeals for the Federal Circuit and its predecessor courts.

This revision does not constitute substantive rulemaking and hence does not have the force and effect of law. It is designed to assist Office personnel in analyzing claimed subject matter for compliance with substantive law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

These Guidelines are intended to form part of the normal examination process. Thus, where Office personnel establish a *prima facie* case of lack of written description for a claim, a thorough review of the prior art and examination on the merits for compliance with the other statutory requirements, including those of 35 U.S.C. 101, 102, 103, and 112, is to be conducted prior to completing an Office action which includes a rejection for lack of written description. Office personnel are to rely on this revision of the Guidelines in the event of any inconsistent treatment of

issues involving the written description requirement between these Guidelines and any earlier guidance provided from the Office.

#### **I. General Principles Governing Compliance With the "Written Description" Requirement for Applications**

The first paragraph of 35 U.S.C. 112 requires that the "specification shall contain a written description of the invention \* \* \*." This requirement is separate and distinct from the enablement requirement.<sup>1</sup> The written description requirement has several policy objectives. "[T]he 'essential goal' of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed."<sup>2</sup> Another objective is to put the public in possession of what the applicant claims as the invention.<sup>3</sup> The written description requirement of the Patent Act promotes the progress of the useful arts by ensuring that patentees adequately describe their inventions in their patent specifications in exchange for the right to exclude others from practicing the invention for the duration of the patent's term.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.<sup>4</sup> An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention.<sup>5</sup> Possession may be shown in a variety of ways including description of an actual reduction to practice,<sup>6</sup> or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete,<sup>7</sup> or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.<sup>8</sup> A question as to whether a specification provides an adequate written description may arise in the context of an original claim which is not described sufficiently, a new or amended claim wherein a claim limitation has been added or removed, or a claim to entitlement of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c).<sup>9</sup> Compliance with the written description requirement is a question of



fact which must be resolved on a case-by-case basis.<sup>10</sup>

#### A. Original Claims

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed.<sup>11</sup> However, the issue of a lack of adequate written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant had possession of the claimed invention.<sup>12</sup> The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art.<sup>13</sup> This problem may arise where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function.<sup>14</sup> A lack of adequate written description issue also arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process.<sup>15</sup>

#### B. New or Amended Claims

The proscription against the introduction of new matter in a patent application<sup>16</sup> serves to prevent an applicant from adding information that goes beyond the subject matter originally filed.<sup>17</sup> Thus, the written description requirement prevents an applicant from claiming subject matter that was not adequately described in the specification as filed. New or amended claims which introduce elements or limitations which are not supported by the as-filed disclosure violate the written description requirement.<sup>18</sup> While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also recognize the appropriate correction.<sup>19</sup> Deposits made after the application filing date cannot be relied upon to support additions to or correction of information in the application as filed.<sup>20</sup>

Under certain circumstances, omission of a limitation can raise an

issue regarding whether the inventor had possession of a broader, more generic invention.<sup>21</sup> A claim that omits an element which applicant describes as an essential or critical feature of the invention originally disclosed does not comply with the written description requirement.<sup>22</sup>

The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed.<sup>23</sup>

## II. Methodology for Determining Adequacy of Written Description

### A. Read and Analyze the Specification for Compliance With 35 U.S.C. 112, ¶ 1

Office personnel should adhere to the following procedures when reviewing patent applications for compliance with the written description requirement of 35 U.S.C. 112, ¶ 1. The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed;<sup>24</sup> however, with respect to newly added or amended claims, applicant should show support in the original disclosure for the new or amended claims.<sup>25</sup> Consequently, rejection of an original claim for lack of written description should be rare. The inquiry into whether the description requirement is met is a question of fact that must be determined on a case-by-case basis.<sup>26</sup>

#### 1. For Each Claim, Determine What the Claim as a Whole Covers

Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description.<sup>27</sup> The entire claim must be considered, including the preamble language<sup>28</sup> and the transitional phrase.<sup>29</sup> The claim as a whole, including all limitations found in the preamble,<sup>30</sup> the transitional phrase, and the body of the claim, must be sufficiently supported to satisfy the written description requirement.<sup>31</sup>

The examiner should evaluate each claim to determine if sufficient structures, acts, or functions are recited to make clear the scope and meaning of the claim, including the weight to be given the preamble.<sup>32</sup> The absence of definitions or details for well-

established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, ¶ 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

#### 2. Review the Entire Application to Understand How Applicant Provides Support for the Claimed Invention Including Each Element and/or Step

Prior to determining whether the disclosure satisfies the written description requirement for the claimed subject matter, the examiner should review the claims and the entire specification, including the specific embodiments, figures, and sequence listings, to understand how applicant provides support for the various features of the claimed invention.<sup>33</sup> The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed<sup>34</sup> and should include a determination of the field of the invention and the level of skill and knowledge in the art. Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification.<sup>35</sup>

#### 3. Determine Whether There is Sufficient Written Description to Inform a Skilled Artisan That Applicant was in Possession of the Claimed Invention as a Whole at the Time the Application Was Filed

a. Original claims. Possession may be shown in many ways. For example, possession may be shown, *inter alia*, by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention.<sup>36</sup>

A specification may describe an actual reduction to practice by showing

that the inventor constructed an embodiment or performed a process that met all the limitations of the claim and determined that the invention would work for its intended purpose.<sup>37</sup>

Description of an actual reduction to practice of a biological material may be shown by specifically describing a deposit made in accordance with the requirements of 37 CFR 1.801 *et seq.*<sup>38</sup>

An applicant may show possession of an invention by disclosure of drawings<sup>39</sup> or structural chemical formulas<sup>40</sup> that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole. The description need only describe in detail that which is new or not conventional.<sup>41</sup> This is equally true whether the claimed invention is directed to a product or a process.

An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics<sup>42</sup> which provide evidence that applicant was in possession of the claimed invention.<sup>43</sup> *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.<sup>44</sup> What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail.<sup>45</sup> If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.<sup>46</sup>

(1) For each claim drawn to a single embodiment or species:<sup>47</sup>

(a) Determine whether the application describes an actual reduction to practice of the claimed invention.

(b) If the application does not describe an actual reduction to practice, determine whether the invention is complete as evidenced by a reduction to drawings or structural chemical formulas that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole.

(c) If the application does not describe an actual reduction to practice or reduction to drawings or structural chemical formula as discussed above, determine whether the invention has been set forth in terms of distinguishing identifying characteristics as evidenced by other descriptions of the invention that are sufficiently detailed to show that applicant was in possession of the claimed invention.

(i) Determine whether the application as filed describes the complete structure

(or acts of a process) of the claimed invention as a whole. The complete structure of a species or embodiment typically satisfies the requirement that the description be set forth "in such full, clear, concise, and exact terms" to show possession of the claimed invention.<sup>48</sup> If a complete structure is disclosed, the written description requirement is satisfied for that species or embodiment, and a rejection under 35 U.S.C. 112, ¶ 1, for lack of written description must not be made.

(ii) If the application as filed does not disclose the complete structure (or acts of a process) of the claimed invention as a whole, determine whether the specification discloses other relevant identifying characteristics sufficient to describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention.<sup>49</sup>

Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient.<sup>50</sup> Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention.<sup>51</sup> In contrast, for inventions in emerging and unpredictable technologies, or for inventions characterized by factors not reasonably predictable which are known to one of ordinary skill in the art, more evidence is required to show possession. For example, disclosure of only a method of making the invention and the function may not be sufficient to support a product claim other than a

product-by-process claim.<sup>52</sup> Furthermore, disclosure of a partial structure without additional characterization of the product may not be sufficient to evidence possession of the claimed invention.<sup>53</sup>

Any claim to a species that does not meet the test described under at least one of (a), (b), or (c) must be rejected as lacking adequate written description under 35 U.S.C. 112, ¶ 1.

(2) For each claim drawn to a genus:

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see (1)(a), above), reduction to drawings (see (1)(b), above), or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see (1)(c), above).<sup>54</sup>

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be situations where one species adequately supports a genus.<sup>55</sup> What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species *cannot* be achieved by disclosing only one species within the genus.<sup>56</sup> Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces.<sup>57</sup> If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description under 35 U.S.C. 112, ¶ 1.

b. New claims, amended claims, or claims asserting entitlement to the benefit of an earlier priority date or filing date under 35 U.S.C. 119, 120, or

365(c). The examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims.<sup>58</sup> However, when filing an amendment an applicant should show support in the original disclosure for new or amended claims.<sup>59</sup> To comply with the written description requirement of 35 U.S.C. 112, ¶ 1, or to be entitled to an earlier priority date or filing date under 35 U.S.C. 119, 120, or 365(c), each claim limitation must be expressly,<sup>60</sup> implicitly,<sup>61</sup> or inherently<sup>62</sup> supported in the originally filed disclosure.<sup>63</sup> Furthermore, each claim must include all elements which applicant has described as essential.<sup>64</sup>

If the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, ¶ 1, as lacking adequate written description, or in the case of a claim for priority under 35 U.S.C. 119, 120, or 365(c), the claim for priority must be denied.

### III. Complete Patentability Determination Under All Statutory Requirements and Clearly Communicate Findings, Conclusions, and Their Bases

The above only describes how to determine whether the written description requirement of 35 U.S.C. 112, ¶ 1, is satisfied. Regardless of the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of title 35 of the U.S. Code.

Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

#### A. For Each Claim Lacking Written Description Support, Reject the Claim Under Section 112, ¶ 1, for Lack of Adequate Written Description

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary

has been presented by the examiner to rebut the presumption.<sup>65</sup> The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims.<sup>66</sup> In rejecting a claim, the examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:

- (1) Identify the claim limitation at issue; and
- (2) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed. A general allegation of "unpredictability in the art" is not a sufficient reason to support a rejection for lack of adequate written description.

When appropriate, suggest amendments to the claims which can be supported by the application's written description, being mindful of the prohibition against the addition of new matter in the claims or description.<sup>67</sup>

#### B. Upon Reply by Applicant, Again Determine the Patentability of the Claimed Invention, Including Whether the Written Description Requirement Is Satisfied by Reperforming the Analysis Described Above in View of the Whole Record

Upon reply by applicant, before repeating any rejection under 35 U.S.C. 112, ¶ 1, for lack of written description, review the basis for the rejection in view of the record as a whole, including amendments, arguments, and any evidence submitted by applicant. If the whole record now demonstrates that the written description requirement is satisfied, do not repeat the rejection in the next Office action. If the record still does not demonstrate that the written description is adequate to support the claim(s), repeat the rejection under 35 U.S.C. 112, ¶ 1, fully respond to applicant's rebuttal arguments, and properly treat any further showings submitted by applicant in the reply. When a rejection is maintained, any affidavits relevant to the 112, ¶ 1, written description requirement,<sup>68</sup> must be thoroughly analyzed and discussed in the next Office action.

Dated: December 29, 2000.

Q. Todd Dickinson.

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

#### Endnotes

<sup>1</sup> See, e.g., *Vas-Cuth, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991).

<sup>2</sup> *In re Barker*, 359 F.2d 588, 392 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977).

<sup>3</sup> See *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), cert. denied, 523 U.S. 1089 (1998).

<sup>4</sup> See, e.g., *Vas-Cuth, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116. Much of the written description case law addresses whether the specification as originally filed supports claims not originally in the application. The issue raised in the cases is most often phrased as whether the original application provides "adequate support" for the claims at issue or whether the material added to the specification incorporates "new matter" in violation of 35 U.S.C. 132. The "written description" question similarly arises in the interference context, where the issue is whether the specification of one party to the interference can support the newly added claims corresponding to the count at issue, i.e., whether that party can "make the claim" corresponding to the interference count. See, e.g., *Martin v. Mayer*, 823 F.2d 500, 503, 3 USPQ2d 1333, 1335 (Fed. Cir. 1987).

In addition, early opinions suggest the Patent and Trademark Office was unwilling to find written descriptive support when the only description was found in the claims; however, this viewpoint was rejected. See *In re Koller*, 613 F.2d 819, 204 USPQ 702 (CCPA 1980) (original claims constitute their own description); accord *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973); accord *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976) (accord). It is now well accepted that a satisfactory description may be in the claims or any other portion of the originally filed specification. These early opinions did not address the quality or specificity of particularity that was required in the description, i.e., how much description is enough.

<sup>5</sup> *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

<sup>6</sup> An application specification may show actual reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 CFR 1.801 *et seq.* See also *Deposit of Biological Materials for Patent Purposes, Final Rule*, 54 FR 34,864 (August 22, 1989) ("The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 U.S.C. 112, and to provide an antecedent basis for the biological material which either has been or will be deposited before the patent is granted." *Id.* at 34,876. "The description must be sufficient to permit verification that the deposited biological material is in fact that disclosed. Once the



patent issues, the description must be sufficient to aid in the resolution of questions of infringement." *Id.* at 34,880.). Such a deposit is not a substitute for a written description of the claimed invention. The written description of the deposited material needs to be as complete as possible because the examination for patentability proceeds solely on the basis of the written description. See, e.g., *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985). See also 54 FR at 34,880 ("As a general rule, the more information that is provided about a particular deposited biological material, the better the examiner will be able to compare the identity and characteristics of the deposited biological material with the prior art.").

<sup>7</sup> *Pfaff v. Wells Electronics, Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 43 USPQ2d 1641, 1647 (1998); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

<sup>8</sup> See *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it").

<sup>9</sup> A description requirement issue can arise for original claims (see, e.g., *Eli Lilly*, 119 F.3d 1559, 43 USPQ2d 1398) as well as new or amended claims. Most typically, the issue will arise in the context of determining whether new or amended claims are supported by the description of the invention in the application as filed (see, e.g., *In re Wright*, 866 F.2d 422, 9 USPQ2d 1649 (Fed. Cir. 1989)), whether a claimed invention is entitled to the benefit of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c) (see, e.g., *Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998); *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed. Cir. 1993); *In re Ziegler*, 992 F.2d 1197, 1200, 26 USPQ2d 1600, 1603 (Fed. Cir. 1993)), or whether a specification provides support for a claim corresponding to a count in an interference (see, e.g., *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971)).

<sup>10</sup> *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991).

<sup>11</sup> *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims").

<sup>12</sup> See endnote 4.

<sup>13</sup> For example, consider the claim "A gene comprising SEQ ID NO:1." A determination of what the claim as a whole covers may result in a conclusion that specific structures such as a promoter, a coding region, or other elements are included. Although all genes encompassed by this claim share the characteristic of comprising SEQ ID NO:1, there may be insufficient description of those specific structures (e.g., promoters, enhancers, coding regions, and other regulatory elements) which are also included.

<sup>14</sup> A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying

characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence. For example, even though a genetic code table would correlate a known amino acid sequence with a genus of coding nucleic acids, the same table cannot predict the native, naturally occurring nucleic acid sequence of a naturally occurring mRNA or its corresponding cDNA. Cf. *In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993), and *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995) (holding that a process could not render the product of that process obvious under 35 U.S.C. 103). The Federal Circuit has pointed out that under United States law, a description that does not render a claimed invention obvious cannot sufficiently describe the invention for the purposes of the written description requirement of 35 U.S.C. 112. *Eli Lilly*, 119 F.3d at 1567, 43 USPQ2d at 1405.

*Compare Fonar Corp. v. General Electric Co.*, 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805 (Fed. Cir. 1997) ("As a general rule, where software constitutes part of a best mode of carrying out an invention, description of such a best mode is satisfied by a disclosure of the functions of the software. This is because, normally, writing code for such software is within the skill of the art, not requiring undue experimentation, once its functions have been disclosed. \* \* \* Thus, flow charts or source code listings are not a requirement for adequately disclosing the functions of software.").

<sup>15</sup> See, e.g., *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996) (a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species); *In re Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967) ("If n-propylamine had been used in making the compound instead of n-butylamine, the compound of claim 13 would have resulted. Appellants submit to us, as they did to the board, an imaginary specific example patterned on specific example 6 by which the above butyl compound is made so that we can see what a simple change would have resulted in a specific supporting disclosure being present in the present specification. The trouble is that there is no such disclosure, easy though it is to imagine it.") (emphasis in original); *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("the specification does not clearly disclose to the skilled artisan that the inventors \* \* \* considered the [ ] ratio to be part of their invention \* \* \*. There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion").

<sup>16</sup> 35 U.S.C. §§ 132 and 251. See also *In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981). See Manual of Patent Examining Procedure (MPEP) §§ 2163.06-2163.07 (7th Ed., Rev. 1, Feb. 2000) for a more detailed discussion of the written description requirement and its relationship to new matter.

<sup>17</sup> The claims as filed in the original specification are part of the disclosure and, therefore, if an application as originally filed contains a claim disclosing material not found in the remainder of the specification, the applicant may amend the specification to include the claimed subject matter. *In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985).

<sup>18</sup> See, e.g., *In re Lukach*, 442 F.2d 967, 169 USPQ 795 (CCPA 1971) (subgenus range was not supported by generic disclosure and specific example within the subgenus range); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily described by a genus encompassing it and a species upon which it reads).

<sup>19</sup> *In re Oda*, 443 F.2d 1200, 170 USPQ 260 (CCPA 1971). With respect to the correction of sequencing errors in applications disclosing nucleic acid and/or amino acid sequences, it is well known that sequencing errors are a common problem in molecular biology. See, e.g., Peter Richterich, *Estimation of Errors in 'Raw' DNA Sequences: A Validation Study*, 8 Genome Research 251-59 (1998). If an application as filed includes sequence information and references a deposit of the sequenced material made in accordance with the requirements of 37 CFR § 1.801 *et seq.*, amendment may be permissible.

<sup>20</sup> Corrections of minor errors in the sequence may be possible based on the argument that one of skill in the art would have resequenced the deposited material and would have immediately recognized the minor error. Deposits made after the filing date can only be relied upon to provide support for the correction of sequence information if applicant submits a statement in compliance with 37 CFR § 1.804 stating that the biological material which is deposited is a biological material specifically defined in the application as filed.

<sup>21</sup> See, e.g., *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473, 45 USPQ2d 1498 (Fed. Cir. 1998) (claims to a sectional sofa comprising, *inter alia*, a console and a control means were held invalid for failing to satisfy the written description requirement where the claims were broadened by removing the location of the control means.); *Johnson Worldwide Associates v. Zebco Corp.*, 175 F.3d 985, 993, 50 USPQ2d 1607, 1613 (Fed. Cir. 1999) (*In Gentry Gallery*, the "court's determination that the patent disclosure did not support a broad meaning for the disputed claim terms was premised on clear statements in the written description that described the location of a claim element—the 'control means'—as 'the only possible location' and that variations were 'outside the stated purpose of the invention.' *Gentry Gallery*, 134 F.3d at 1479, 45 USPQ2d at 1503. *Gentry Gallery*, then, considers the situation where the patent's disclosure makes crystal clear that a particular (*i.e.*, narrow) understanding of a claim term is an 'essential element of [the inventor's] invention.'"); *Tronzo v. Biomet*, 156 F.3d at 1158-59, 47 USPQ2d at 1833 (Fed. Cir. 1998) (claims to generic cup shape were not entitled to filing date of parent application which disclosed "conical cup" in view of the disclosure of the

parent application stating the advantages and importance of the conical shape.).

<sup>22</sup> See *Gentry Gallery*, 134 F.3d at 1480, 45 USPQ2d at 1503; *In re Sus*, 306 F.2d 494, 504, 134 USPQ 301, 309 (CCPA 1962) ("[O]ne skilled in this art would not be taught by the written description of the invention in the specification that any 'aryl or substituted aryl radical' would be suitable for the purposes of the invention but rather that only certain aryl radicals and certain specifically substituted aryl radicals (i.e., aryl azides) would be suitable for such purposes.") (emphasis in original). A claim which omits matter disclosed to be essential to the invention as described in the specification or in other statements of record may also be subject to rejection under 35 U.S.C. 112, ¶ 1, as not enabling, or under 35 U.S.C. 112, ¶ 2. See *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976); *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976); and *In re Collier*, 397 F.2d 1003, 158 USPQ 266 (CCPA 1968). See also MPEP § 2172.01.

<sup>23</sup> See, e.g., *Vas-Cath, Inc.*, 935 F.2d at 1563-64, 19 USPQ2d at 1117.

<sup>24</sup> *Wertheim*, 541 F.2d at 262, 191 USPQ at 96.

<sup>25</sup> See MPEP §§ 714.02 and 2163.06 ("Applicant should \* \* \* specifically point out the support for any amendments made to the disclosure."); and MPEP § 2163.04 ("If applicant amends the claims and points out where and/or how the originally filed disclosure supports the amendment(s), and the examiner finds that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of the filing of the application, the examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

<sup>26</sup> See *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) ("Precisely how close [to the claimed invention] the description must come to comply with § 112 must be left to case-by-case development."); *In re Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (inquiry is primarily factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure).

<sup>27</sup> See, e.g., *In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997).

<sup>28</sup> "Preamble language" is that language in a claim appearing before the transitional phrase, e.g., before "comprising," "consisting essentially of," or "consisting of."

<sup>29</sup> The transitional term "comprising" (and other comparable terms, e.g., "containing," "including," and "having") is "open-ended—it covers the expressly recited subject matter, alone or in combination with unrecited subject matter. See, e.g., *Genentech, Inc. v. Chiron Corp.*, 112 F.3d 495, 501, 42 USPQ2d 1608, 1613 (Fed. Cir. 1997) ("'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim."); *Ex parte Davis*, 80 USPQ 448, 450 (Bd. App. 1948) ("comprising" leaves the

"claim open for the inclusion of unspecified ingredients even in major amounts"). "By using the term 'consisting essentially of,' the drafter signals that the invention necessarily includes the listed ingredients and is open to unlisted ingredients that do not materially affect the basic and novel properties of the invention. A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." *PPG Industries v. Guardian Industries*, 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353-54 (Fed. Cir. 1998). For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, 'consisting essentially of' will be construed as equivalent to 'comprising.' See, e.g., *PPG*, 156 F.3d at 1355, 48 USPQ2d at 1355 ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics of the invention."). See also *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 895-96 (CCPA 1963). If an applicant contends that additional steps or materials in the prior art are excluded by the recitation of "consisting essentially of," applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964).

<sup>30</sup> See *Pac-Tec Inc. v. Amerace Corp.*, 903 F.2d 796, 801, 14 USPQ2d 1871, 1876 (Fed. Cir. 1990) (determining that preamble language that constitutes a structural limitation is actually part of the claimed invention).

<sup>31</sup> An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations. *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

<sup>32</sup> See, e.g., *Bell Communications Research, Inc. v. Vitalink Communications Corp.*, 55 F.3d 615, 620, 34 USPQ2d 1816, 1820 (Fed. Cir. 1995) ("[A] claim preamble has the import that the claim as a whole suggests for it."); *Corning Glass Works v. Sumitomo Elec. U.S.A., Inc.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989) (The determination of whether preamble recitations are structural limitations can be resolved only on review of the entirety of the application "to gain an understanding of what the inventors actually invented and intended to encompass by the claim.").

<sup>33</sup> An element may be critical where those of skill in the art would require it to determine that applicant was in possession of the invention. *Compare Rasmussen*, 650 F.2d at 1215, 211 USPQ at 327 ("one skilled in the art who read Rasmussen's specification would understand that it is unimportant how the layers are adhered, so long as they are adhered") (emphasis in original). *With Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) ("it is well established in our law that conception of a chemical

compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it").

<sup>34</sup> See, e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993).

<sup>35</sup> See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 302 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

<sup>36</sup> See, e.g., *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, \_\_\_, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) (the written description "inquiry is a factual one and must be assessed on a case-by-case basis"); see also *Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 311, 48 USPQ2d at 1646 ("The word 'invention' must refer to a concept that is complete, rather than merely one that is 'substantially complete.' It is true that reduction to practice ordinarily provides the best evidence that an invention is complete. But just because reduction to practice is sufficient evidence of completion, it does not follow that proof of reduction to practice is necessary in every case. Indeed, both the facts of the *Telephone Cases* and the facts of this case demonstrate that one can prove that an invention is complete and ready for patenting before it has actually been reduced to practice.").

<sup>37</sup> *Cooper v. Goldfarb*, 154 F.3d 1321, 1327, 47 USPQ2d 1896, 1901 (Fed. Cir. 1998). See also *UMC Elecs. Co. v. United States*, 816 F.2d 647, 652, 2 USPQ2d 1465, 1468 (Fed. Cir. 1987) ("[T]here cannot be a reduction to practice of the invention \* \* \* without a physical embodiment which includes all limitations of the claim."); *Estee Lauder Inc. v. L'Oreal, S.A.*, 129 F.3d 588, 593, 44 USPQ2d 1610, 1614 (Fed. Cir. 1997) ("[A] reduction to practice does not occur until the inventor has determined that the invention will work for its intended purpose."); *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1578, 38 USPQ2d 1288, 1291 (Fed. Cir. 1996) (determining that the invention will work for its intended purpose may require testing depending on the character of the invention and the problem it solves).

<sup>38</sup> 37 CFR 1.804, 1.809. See also endnote 6.

<sup>39</sup> See, e.g., *Vas-Cath*, 935 F.2d at 1565, 19 USPQ2d at 1118 ("drawings alone may provide a 'written description' of an invention as required by § 112"); *In re Wolfensperger*, 302 F.2d 950, 133 USPQ 537 (CCPA 1962) (the drawings of applicant's specification provided sufficient written descriptive support for the claim limitation at issue); *Autogiro Co. of America v. United States*, 384 F.2d 391, 398, 155 USPQ 697, 703 (Ct. Cl. 1967) ("In those instances where a visual representation can flesh out words, drawings may be used in the same manner and with the same limitations as the specification.").

<sup>40</sup> See, e.g., *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus.").

<sup>41</sup> See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805 (source code description not required).

<sup>42</sup> For example, the presence of a restriction enzyme map of a gene may be relevant to a statement that the gene has been isolated. One skilled in the art may be able to determine when the gene disclosed is the same as or different from a gene isolated by another by comparing the restriction enzyme map. In contrast, evidence that the gene could be digested with a nuclease would not normally represent a relevant characteristic since any gene would be digested with a nuclease. Similarly, isolation of an mRNA and its expression to produce the protein of interest is strong evidence of possession of an mRNA for the protein.

For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. For example, unique cleavage by particular enzymes, isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities, or antibody cross-reactivity may be sufficient to show possession of the claimed invention to one of skill in the art. See *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966 ("written description" requirement may be satisfied by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention").

<sup>43</sup> A definition by function alone "does not suffice" to sufficiently describe a coding sequence "because it is only an indication of what the gene does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. See also *Fiers*, 984 F.2d at 1169-71, 25 USPQ2d at 1605-06 (discussing *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)).

<sup>44</sup> If a claim limitation invokes 35 U.S.C. 112, ¶ 6, it must be interpreted to cover the corresponding structure, materials, or acts in the specification and "equivalents thereof." See 35 U.S.C. 112, ¶ 6. See also *B. Braun Medical, Inc. v. Abbott Lab.*, 124 F.3d 1419, 1424, 43 USPQ2d 1896, 1899 (Fed. Cir. 1997). In considering whether there is 35 U.S.C. 112, ¶ 1, support for a means- (or step-) plus-function claim limitation, the examiner must consider not only the original disclosure contained in the summary and detailed description of the invention portions of the specification, but also the original claims, abstract, and drawings. A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, ¶ 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-

function limitation. Note also: A rejection under 35 U.S.C. 112, ¶ 2, "cannot stand where there is adequate description in the specification to satisfy 35 U.S.C. 112, first paragraph, regarding means-plus-function recitations that are not, per se, challenged for being unclear." *In re Noll*, 545 F.2d 141, 149, 191 USPQ 721, 727 (CCPA 1976). See *Supplemental Examination Guidelines for Determining the Applicability of 35 U.S.C. 112, ¶ 6*, 65 FR 38510, June 21, 2000.

<sup>45</sup> See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94.

<sup>46</sup> See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., 'in the same words'] to be sufficient").

<sup>47</sup> A claim which is limited to a single disclosed embodiment or species is analyzed as a claim drawn to a single embodiment or species, whereas a claim which encompasses two or more embodiments or species within the scope of the claim is analyzed as a claim drawn to a genus. See also MPEP § 806.04(e).

<sup>48</sup> 35 U.S.C. 112, ¶ 1. Cf. *Fields v. Conover*, 443 F.2d 1386, 1392, 170 USPQ 276, 280 (CCPA 1971) (finding a lack of written description because the specification lacked the "full, clear, concise, and exact written description" which is necessary to support the claimed invention).

<sup>49</sup> For example, if the art has established a strong correlation between structure and function, one skilled in the art would be able to predict with a reasonable degree of confidence the structure of the claimed invention from a recitation of its function. Thus, the written description requirement may be satisfied through disclosure of function and minimal structure when there is a well-established correlation between structure and function. In contrast, without such a correlation, the capability to recognize or understand the structure from the mere recitation of function and minimal structure is highly unlikely. In this latter case, disclosure of function alone is little more than a wish for possession; it does not satisfy the written description requirement. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 (written description requirement not satisfied by merely providing "a result that one might achieve if one made that invention"); *In re Wilder*, 736 F.2d 1516, 1521, 222 USPQ 369, 372-73 (Fed. Cir. 1984) (affirming a rejection for lack of written description because the specification does "little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate"). Compare *Fonar*, 107 F.3d at 1549, 41 USPQ2d at 1805 (disclosure of software function adequate in that art).

<sup>50</sup> See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

<sup>51</sup> See, e.g., *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) ("One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure

obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.")

<sup>52</sup> See, e.g., *Fiers v. Revel*, 984 F.2d at 1169, 25 USPQ2d at 1605; *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021. Where the process has actually been used to produce the product, the written description requirement for a product-by-process claim is clearly satisfied; however, the requirement may not be satisfied where it is not clear that the acts set forth in the specification can be performed, or that the product is produced by that process.

<sup>53</sup> See, e.g., *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021 ("A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated.") (citations omitted). In such instances the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor's idea of the invention. *Burroughs Wellcome Co. v. Barr Laboratories Inc.*, 40 F.3d 1223, 1229, 32 USPQ2d 1915, 1920 (Fed. Cir. 1994). Reduction to practice in effect provides the only evidence to corroborate conception (and therefore possession) of the invention. *Id.*

<sup>54</sup> See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

<sup>55</sup> See, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27 (disclosure of a single method of adhering by applying one layer to another was sufficient to support a generic claim to "adhering by applying" because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered); *In re Herschler*, 591 F.2d 693, 697, 200 USPQ 711, 714 (CCPA 1979) (disclosure of corticosteroid in DMSO sufficient to support claims drawn to a method of using a mixture of a "physiologically active steroid" and DMSO because "use of known chemical compounds in a manner auxiliary

to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description."'); *In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 285 (CCPA 1973) (the phrase "air or other gas which is inert to the liquid" was sufficient to support a claim to "inert fluid media" because the description of the properties and functions of the air or other gas segmentizing medium would suggest to a person skilled in the art that appellant's invention includes the use of "inert fluid" broadly.). However, in *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833 (Fed. Cir. 1998), the disclosure of a species in the parent application did not suffice to provide written description support for the genus in the child application.

<sup>56</sup> See, e.g., *Eli Lilly*.

<sup>57</sup> For example, in the molecular biology arts, if an applicant disclosed an amino acid sequence, it would be unnecessary to provide an explicit disclosure of nucleic acid sequences that encoded the amino acid sequence. Since the genetic code is widely known, a disclosure of an amino acid sequence would provide sufficient information such that one would accept that an applicant was in possession of the full genus of nucleic acids encoding a given amino acid sequence, but not necessarily any particular species. Cf. *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994).

<sup>58</sup> See *Wertheim*, 541 F.2d at 263, 191 USPQ at 97 ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

<sup>59</sup> See MPEP §§ 714.02 and 2163.06 ("Applicant should \* \* \* specifically point out the support for any amendments made to the disclosure.").

<sup>60</sup> See, e.g., *In re Wright*, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989) (Original specification for method of forming images using photosensitive microcapsules which describes removal of microcapsules from surface and warns that capsules not be disturbed prior to formation of image, unequivocally teaches absence of permanently fixed microcapsules and supports amended language of claims requiring that microcapsules be "not permanently fixed" to underlying surface, and therefore meets description requirement of 35 U.S.C. 112.).

<sup>61</sup> See, e.g., *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification \* \* \* mention of representative compounds may provide an implicit description upon which to base generic claim language."'); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily implicitly described by a genus encompassing it and a species upon which it reads).

<sup>62</sup> See, e.g., *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir.

1999) ("To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient."') (citations omitted).

<sup>63</sup> When an explicit limitation in a claim "is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation." *Hyatt v. Boone*, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998).

<sup>64</sup> See, e.g., *Johnson Worldwide Associates Inc. v. Zebco Corp.*, 175 F.3d at 993, 50 USPQ2d at 1613; *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d at 1479, 45 USPQ2d at 1503; *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833.

<sup>65</sup> See, e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

<sup>66</sup> *Wertheim*, 541 F.2d at 263, 191 USPQ at 97.

<sup>67</sup> See *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326.

<sup>68</sup> See *In re Alton*, 76 F.3d 1168, 1176, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996).

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## CORPORATION FOR NATIONAL AND COMMUNITY SERVICE

### Revision of Currently Approved Information Collection; Comment Request

AGENCY: Corporation for National and Community Service

ACTION: Notice.

**SUMMARY:** The Corporation for National and Community Service (hereinafter "Corporation"), as part of its continuing effort to reduce paperwork and respondent burden, conducts a preclearance consultation program to provide the general public and Federal agencies with an opportunity to comment on proposed and/or continuing collections of information in accordance with the Paperwork Reduction Act of 1995 (PRA95) (44 U.S.C. 3506(c)(2)(A)). This program helps to ensure that requested data can be provided in the desired format, reporting burden (time and financial resources) is minimized, collection instruments are clearly understood, and the impact of collection requirement on respondents can be properly assessed.

Currently, the Corporation is soliciting comments concerning the proposed revision of its Voucher and

Payment Request Form (OMB #3045-0014).

Copies of the forms can be obtained by contacting the office listed below in the address section of this notice.

**DATES:** Written comments must be submitted to the office listed in the ADDRESSES section by March 6, 2001.

**ADDRESSES:** Send comments to Levon Buller, National Service Trust, Corporation for National and Community Service, 1201 New York Ave., NW., Washington, DC 20525.

**FOR FURTHER INFORMATION CONTACT:** Levon Buller, (202) 606-5000, ext. 383.

**SUPPLEMENTARY INFORMATION:** The Corporation is particularly interested in comments which:

- Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the Corporation, including whether the information will have practical utility;
- Evaluate the accuracy of the agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;
- Enhance the quality, utility and clarity of the information to be collected; and
- Minimize the burden of the collection of information on those who are to respond, including through the use of appropriate automated, electronic, mechanical, or other technological collection techniques or other forms of information technology, e.g., permitting electronic submissions of responses.

### Background

The Corporation supports programs that provide opportunities for individuals who want to become involved in national service. The service opportunities cover a wide range of activities over varying periods of time. Upon successfully completing an agreed-upon term of service in an approved AmeriCorps program, a national service participant—an AmeriCorps member—receives an "education award". This award is an amount of money set aside in the member's name in the National Service Trust Fund. This education award can be used to make payments towards qualified student loan or pay for educational expenses at qualified post-secondary institutions and approved school-to-work opportunities programs. Members have seven years in which to draw against any unused balance.

The National Service Trust is the office within the Corporation that administers the education award

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